Decreasing the Prevalence of Foot Pad Dermatitis in Commercial Turkeys

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Declaration

I declare that the work described in this thesis is my own composition and no part of it has been presented in any other thesis or work. The work presented was carried out by myself, unless otherwise stated.

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Dedication

I would like to dedicate this work to my parents Geoff and Lesley, who encouraged my love of science in the first place. I would also like to dedicate this to my husband Andrew, without whom I would have lost the enthusiasm for science a long time ago. Thank you.

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Publications

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Abstract: Decreasing the Prevalence of Foot Pad Dermatitis in Commercial Turkeys

The results from the work carried out as part of this PhD have led to the conclusion that wetness of the litter can induce severe FPD lesions in young turkeys within a very short space of time (less than 48 h) in the absence of significant excreta, regardless of litter type, when compared with birds housed on dry litter. Previous to this work, no model had been reported that enabled FPD to be induced. These experiments provided a method by which FPD could be induced rapidly, which allowed for observations and measurements to be made. When birds were housed on wet litter, inflammatory responses became apparent after just 24 h and the severity of the lesions increased with time. The use of this model has been vital to understanding the development of FPD. The practical outcome of this research is that litter must be better managed to improve turkey welfare and decrease the prevalence of FPD.

Cell types involved in the development of FPD were identified under the microscope using H & E stain; notably heterophils and eosinophils. Other cells identified were macrophages and T cells (CD4+ and CD8+) which were observed using immunohistochemical staining. The presence of these cells in birds affected by FPD suggested that there was an inflammatory response occurring within the skin of the foot pad. qRT-PCR techniques were employed to identify cytokine expression within turkey foot pad skin. The most notable differences were between the expression of IFN-γ, IL-1ß, IL-6 and IL-8. The expression of these cytokines was

considerably greater in birds housed on wet litter when compared with those housed on dry litter. The higher expressions of IL-1ß, IL-6 and IL-8 are consistent with an inflammatory rather than an allergic response. From these data it can be concluded that the reaction occurring in the birds as a result of FPD is an inflammatory one, with no definite evidence of a cell mediated allergic response to an environmental factor.

List of Abbreviations

Abbreviation used	Full definition
B.U.T	British United Turkeys
B.N.F	Buffered neutral formalin
d	Days
df	Degrees of freedom
FPD	Foot Pad Dermatitis
h	Hours
IL-	Interleukin
I.A.H	Institute for Animal Health
H & E stain	Haemotoxylin & Eosin stain
l	Litres
min	Minutes
PCR	Polymerase Chain Reaction
ppm	Parts per million
qRT-PCR	Quantitative Reverse Transcriptase - Polymerase Chain Reaction
RT-PCR	Reverse Transcriptase - Polymerase Chain Reaction
SED	Standard Error of the Difference
Wk/wks	Week/weeks

CONTENTS

Chapter 1

A review of the aetiology of foot pad dermatitis in growing turkeys and broilers

Summary

Foot pad dermatitis (FPD) is a common condition amongst commercially grown turkey poults. It causes the skin of the footpad to become hard and scaly, often developing horn-like pegs. The footpad can become swollen, and necrosis of the integument occurs. The skin of the footpad frequently splits. In the centre of the lesion (on the footpad), the epidermis separates, and is often totally necrotic. Heterophils infiltrate the stratum germinativum. Abnormal keratin formation may also be evident.

The cause of FPD is unknown, but many contributing factors have been suggested such as skin structure; sex; the amount or concentration of biotin, methionine, zinc, pantothenic acid, lysine, manganese, riboflavin and vitamin G in the diet; the weight and size of the bird; litter moisture; litter pH and ammonia content; and litter type. Litter quality is affected by many other factors such as stocking density, air temperature and moisture, season, consistency and amount of faeces (affected by diet), and drinker design.

Experimental evidence suggests that biotin deficiency causes FPD, and that commercial rations do not contain enough biotin to prevent these lesions. Supplementations of biotin between 250-440 μg/kg of feed have been shown to reduce the severity and incidence of lesions. In one experiment 2000 μg/kg of feed prevented lesions. Wet litter has also been identified as an important causative agent. Broilers and poults reared on wet litter have an increased incidence and severity of

FPD lesions, but the problem is alleviated by replacing the wet litter with dry. Biotin supplementations are able to prevent FPD to a certain extent, if reared on dry litter, but supplementations are not as effective on wet litter.

Experimental results are difficult to compare because rearing conditions differ. Further experimentation is needed to determine the optimum amount of biotin required for healthy growth and lesion-free feet, and to ascertain the real effects of other suggested causes.

Introduction

The prevalence of footpad dermatitis (FPD) in commercially reared turkeys is extremely high. Although there are various estimates of its prevalence, it is difficult to compare findings because the scoring systems used in different experiments are not the same. The USA claim to have 70 % in hens, and 78 % in stags (Paulus 2002). The UK has 67 % prevalence in hens, and 83 % in stags (Paulus 2002). However, this difference between the USA and the UK may be due to different bedding materials available and external temperatures between these countries. FPD has been reported to be a common occurrence in commercial turkey production (Ekstrand *et al.* 1997; Berg 1998). In a survey carried out by Ekstrand & Algers (1997) 98 % of Swedish turkey poults had evidence of FPD. Berg (1998) estimated the prevalence of FPD in Swedish turkeys to be 20 % for severe lesions (ulcers) and 78 % for mild lesions (discolouration, erosion). A survey of the UK turkey industry was carried out by a major turkey producer in the UK (see Appendix 1). The data has not been published, but the raw data were analysed to provide a general overview of the problem of FPD. These data highlight the high prevalence of the problem within the UK commercial turkey industry (~90 % of birds affected) and are the basis for the current research.

In the UK, several scoring systems are in use, one of these is the Martland UK system for scoring FPD specifically in turkeys, this is based on the work of Martland (1984). The same scale was later used to score broiler chickens for FPD (Martland

1985). Both hens and stags are scored on the same scale, but since stags have been observed to have more severe lesions, a score of 3 is reserved only for stags.

Table 1.1. Scoring system devised by Martland (1984) to classify turkey foot pad dermatitis

Score	Lesion
0	None
1	Small scab(s) <5% pad area
2	Larger scabs <25% pad area
3(stags only)	Severe, large scab-filled ulcers

Table 1.2. Martland's scoring system was adapted by Clark, (2002) to describe skin condition on the foot rather than classifying lesions according to size

Score	External signs
0	Normal
1	Redness & slight necrosis of foot pad (blackening)
2	Moderate necrosis of foot pad spreading to digits (toes)
3 (stags only)	Severe necrosis of foot pad & digits

Figure 1.1. Visual representation of turkey foot pad classification by Clark's scoring system extending the scoring system in Table1.2. (Clark 2002; Clark *et al.* 2002)

Table 1.3. A scoring system devised and first reported in 1994 (Ekstrand 1994)[1] divided lesions into 6 separate categories to provide a more detailed scoring system than both Marland's or Clark's

Score	Description of foot pad
1	No visible lesions: smooth epidermis, no discolouration
2	Papillae only: hyperkeratosis but no discolouration
3	Mild/superficial lesions: discolouration or erosions in the epidermal layer
4	Mild/superficial lesions and papillae: hyperkeratosis and discolouration or erosions in the epidermal layer
5	Severe ulcerations: discolourations, ulcers and signs of inflammatory rections
6	Severe ulcerations and papillae: discolouration, hyperkeratosis, ulcers and signs of inflammatory reactions

The supermarket, Tesco plc, have their own visual comparison chart, as do the commercial turkey production company, Bernard Matthews. Both charts categorise foot pad lesions according to lesion size.

1. External description of the condition

Poultry are often observed to have discoloured areas of skin on the foot, and slight lesions or ulcers of the footpad. These may appear similar externally, regardless of cause. Turkey poults exhibit dermatitis of the foot in response to a vitamin G (now known as riboflavin) deficiency, whilst chicks show similar symptoms in response to a deficiency of 'filtrate factor' (Lepkovsky & Jukes 1936a). Lepkovsky & Jukes

[1] Ekstrand's scoring system was first published in German in 1994, but was reproduced in later papers in English.

believed that the filtrates from liver extract and rice bran were thought to contain B vitamins that were essential in the prevention of FPD. The lesions of the feet may look the same, yet the cause is different. When foot lesions occur, hyperplasia of the epidermis takes place, inflammation and ulceration of the pad occurs, causing an enlargement of the foot (Pass 1989).

FPD causes the skin of the metatarsal pad and digital pads to become hard, scaly, discoloured, and sometimes swollen. Greene *et al.* (1985) reported brown or black erosions and ulcers, along with acute inflammation and necrosis of the epidermis and in severe cases, necrosis of the upper dermis of the footpad. Small scabs (hard discoloured areas of skin) appeared at first on the metatarsal pad. These usually began as cracks, abrasions and small brown scabs on the surface of the footpad that developed progressively over the first few weeks of life. In a few cases, a severe ulcer developed within a week (Greene *et al.* 1985). These scabs spread as time progressed to cover a larger area of the metatarsal pad until they occupied a large proportion of it, and often several digital pads as well. Swelling sometimes accompanied more severe cases of FPD.

Greene *et al.* (1985) observed different types of lesions. If the scab could be peeled off easily, along with some superficial epidermis, but leaving the basal layer of epidermis intact, this was classified as an 'erosion'. More severe cases were classified as 'ulcers' if the footpad showed an ulcer filled with congealed exudate. An ulcer was accompanied by subcutaneous inflammation, resulting in red swollen skin around the site of injury.

2. Histopathological changes that occur within the foot pad

It was usually the weight bearing surfaces of growing birds that exhibited the cellular changes characteristic of FPD (Platt *et al.* 2003) and hyperplasia of the epidermis and hyperkeratosis were often observed (Whitehead 1990). On the surface of the foot pad, plant material and bacteria may be present, but were seldom found in deeper layers (Martland 1985; Greene *et al.* 1985).

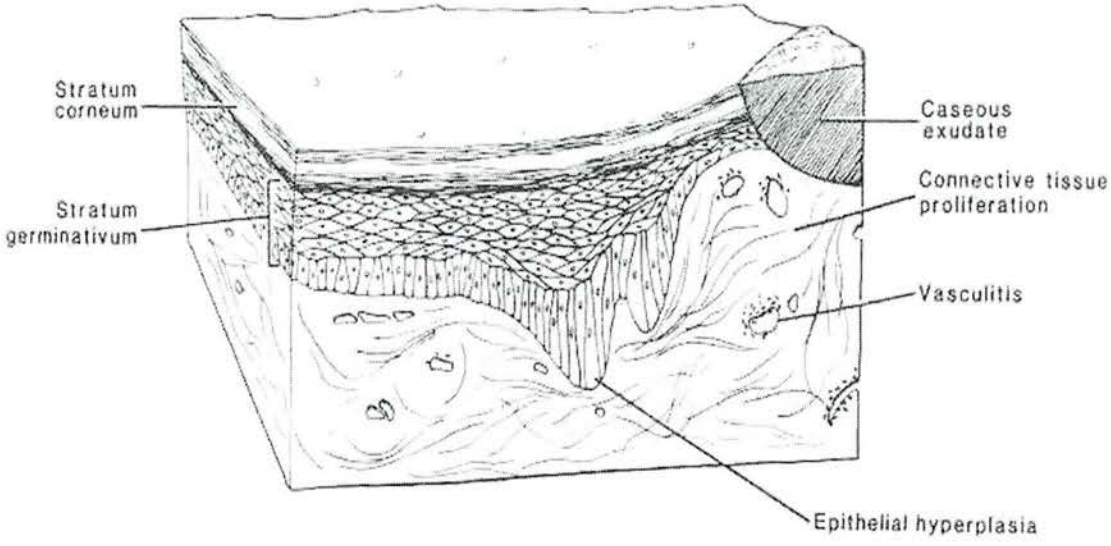
Whitehead (1977) recorded the progression of the condition in turkey poults. He observed dermatitis initially as scaly skin on the foot pad, progressing into cracks, with haemorrhaging and scabs developing on the foot pad and digital pads.

More recently, Platt *et al.* (2001) also studied the development of foot lesions in turkey poults. At 6 weeks of age hyperkeratosis of the footpads was observed. Keratin layers were becoming separated. Lesions were mostly superficial, but sometimes spread from the epidermis into the dermis, developing into ulcers. The number of lymphocytes and granulocytes, as well as lymph follicles increased within the dermis adjacent to the lesions. At 14 weeks of age, the prevalence of superficial lesions decreased, whilst ulceration increased. Keratin layers become separated and hyperkeratosis occurred.

Gonder & Barnes (1987) highlighted some of the changes that take place at a cellular level when a footpad lesion occurs (Fig 1.2.). The stratum corneum, also known as the keratin layer, is the external covering of the foot pad. The stratum germinativum

sits below the stratum corneum, producing new cells for the epithelium. The epithelium lies on the lower surface of the stratum germinativum. When a lesion occurs, thick and sticky exudate may force its way upwards through these upper layers and be evident on the surface of the footpad. The epithelium may become thickened due to an increased number of cells; this is known as epithelial hyperplasia. There may also be a proliferation of connective tissue that lies around the cells below the epithelium. Vasculitis may result, with blood vessels becoming inflamed and necrotic.

Figure 1.2. Diagrammatical representation of the turkey integument exhibiting a lesion (Gonder & Barnes 1987)



The mildest lesions showed simply an infiltration of heterophils into the stratum germinativum, and defects in keratin formation (Martland 1984). An infiltration of heterophils into the dermis, and sub-epidermis, as well as a few heterophils in the epidermis, was seen to occur (Greene *et al.* 1985). Also observed was evidence of

basophilic debris in the keratin layer, and stratum corneum (outer keratin layer). After these cells had performed their function, they were no longer useful; the cells disintegrated and the debris could be seen within the keratin layers of the surface of the footpad (Greene *et al.* 1985). Small vacuoles (often containing heterophils) were also seen in the epidermis (Greene *et al.* 1985). Heterophils had infiltrated the dermis and inside blood vessels, leucocytosis had occurred (Greene *et al.* 1985; Martland 1984, Martland 1985; Harms & Simpson 1975). In the centre of the lesion, there was often complete destruction of the epidermis, including the keratin; in its place was a mass of eosinophils and basophilic nuclear debris. Underneath, necrosis had occurred, and there was mass of inflammatory cells (mostly heterophils). Occasionally, thrombosis and necrosis of blood vessels was noted (Greene *et al.* 1985).

In more severe, ulcerated lesions the major observation was acute inflammation. Capillaries in the dermis became congested, and hyperplasia of the epidermis at the edges of the lesion was observed (Greene *et al.* 1985). More dense cellular infiltration occurred, and there were more obvious defects in the stratum corneum (Martland 1984). The epidermis had been eroded, exposing inflammatory cells, and necrotic debris. The dermis had become filled with fluid and inflammatory cells and blood vessels had become dilated (Whitehead 1990). The keratin layer also became thicker and formed itself into 'horned pegs' (Martland 1984; Whitehead 1990). These 'horned pegs' were knobs of hard keratin that protruded upwards from the surface keratin. Foot lesions observed in older turkeys appeared different from those seen in younger birds. Younger birds showed scaliness and/or thickening and

cracking of the skin, whilst older birds also exhibited an enlarged ball of flesh within the footpad that sometimes included scar tissue (Richardson & Wilgus 1967).

FPD has been noted in broiler chickens as well as turkeys (Patrick *et al.* 1942; Hill 1975; Harms *et al.* 1977; Scott 1981; Roush & Arscott 1983; Burger & Arscott 1984; Martland 1985; Bruce *et al.* 1990; Tauson & Abrahamsson 1994; Ekstrand *et al.* 1997; Ekstrand *et al.* 1998; Wang *et al.* 1998; Ekstrand & Carpenter 1998a; Ekstrand & Carpenter 1998b; Ekstrand & Carpenter 1998c). Although the lesions observed in turkeys and chickens were similar, Martland (1985) observed that in chickens, the metatarsal and digital pads, as well as the interpad spaces became ulcerated, whilst only the pads showed lesions in turkeys.

3. Possible causes of FPD

At present the cause of FPD is unknown. Many singular factors (that will be reviewed below) have been suggested, but it is more likely that the cause is multifactorial. It may be that FPD is affected by an internal factor, such as skin structure, the sex of the bird, the bird's bodyweight, diet (for example, the amount of amino acids and vitamins provided). All these factors may affect skin structure.

However, FPD may also be affected by external factors, such as the wetness of the litter. This may in turn be affected by the drinker design, the consistency and amount of excreta (which is affected by diet), humidity (affected by the season, and stocking density), the pH of the litter, and the amount of ammonia and other irritants within the litter. The type of the litter, such as woodchips, bare concrete, or wire floors may also affect the development of FPD.

Most work has been carried out in broiler chickens rather than turkeys, but it has been assumed that the causative agent of FPD will be the same for turkeys as for chickens, as the skin structure and environmental conditions for commercially bred chickens and turkeys are similar (Pass 1989).

Ekstrand has published a number of papers highlighting the rearing conditions, and prevalence of FPD in both broilers and turkeys in Sweden. She suggested that FPD is a contact dermatitis that begins with hyperkeratosis, erosion and discolouration of the skin. Erosions then develop into ulcers. Altered management techniques may

prevent the condition. Ekstrand concluded that the major factors that affected the development of FPD were wet litter (associated with drinker type), litter depth, and litter material.

FPD has been associated with the presence of breast lesions (Gonder & Barnes 1987; Harms & Simpson 1975), focal ulcerative dermatitis (FUD) (Kamyab 2001) and hock burns in both broilers and turkeys (Bruce *et al.* 1990). From reports of breast, hock and footpad lesions, the pathology of each appeared similar to a contact dermatitis (Gonder & Barnes 1987; Greene *et al.* 1985; Martland 1984). Breast lesions had a central scab composed histologically of exudated protein and necrotic inflammatory cells. This was sometimes covered by cornified strands of keratin. This description is similar to lesions seen on the footpads of poultry.

3.1. Internal factors

3.1.1) Breed

Large White turkey poults were found to be more susceptible to FPD than Broad Breasted Bronze poults when reared in the same conditions on wire floors (Chavez & Kratzer 1972). Using a scoring system of 0 for a normal foot pad, and 4 for a severe lesion, Large White poults had an average score of 0.60, whilst Bronze poults had a score of 0.20 (Chavez & Kratzer 1972). However, this may be due to the fact that Large White poults had a more rapid rate of growth than Bronze poults, resulting in heavier birds.

Roush & Arscott (1983) reported that the incidence of FPD was closely related to the strain of birds tested. Dwarf hens had a much higher incidence than did normal hens despite the fact that both were Single Comb White Leghorn layers. Swedish Ross chicks were found to have a significantly lower prevalence of FPD than Danish Ross chicks (Sanotra *et al.* 2003). However, this may be due to the fact that a higher litter quality was recorded in houses containing Swedish Ross chicks.

3.1.2) Diet

Many substances have been hypothesised to be involved in skin formation and maintenance. These include vitamins and amino acids such as biotin, pantothenic acid, methionine, zinc, lysine, manganese, riboflavin (vitamin G).

If the weight of the bird is linked to the prevalence of FPD (Harms & Simpson 1982), total calorific content of the feed, and any substances that influence growth rate will have an effect on FPD.

3.1.2.1 The role of Biotin in avian species

Biotin deficiency results in a dermatitis that appears first on the foot pads (Patrick *et al.* 1942). D-biotin is the active form of the vitamin, whilst L-biotin, is not active (Scott 1981). Biotin is required in carbohydrate metabolism, fatty acid synthesis, protein synthesis (through its effects on ribonucleic acid formation), amino acid synthesis, amino acid deamination, purine synthesis, and nucleic acid metabolism formation (Wakeman 2002; Whitehead 1977). Because biotin is needed for the vital basic functions, it has an effect on most major systems, especially the cutaneous

system. Biotin deficiency may also reduce growth and feed efficiency. Biotin can, however be synthesised by intestinal micro-organisms, but such naturally occurring biotin is usually bound to other molecules (e.g. avidin in egg white) (Wakeman 2002), and is not available for use by the bird.

3.1.2.2 Biotin deficiency symptoms

Biotin deficiency is often observed in growing birds (although it is not often seen before 10 days of age) and causes external symptoms such as depressed growth (Patrick *et al.* 1942; Wakeman 2002), poor food conversion rate (Patrick *et al.* 1942; Oloyo 1991; Wakeman 2002), disturbed feathering (Wakeman 2002), skin lesions (Wakeman 2002), dry and flaky skin of the feet (Wakeman 2002), foot pad lesions (Patrick *et al.* 1942; Whitehead & Bannister 1981; Whitehead 1990; Oloyo 1991; Wakeman 2002), ulceration and cracking of the foot pad (Whitehead & Bannister 1981), lesions on the eyelids, beak, and vent (Whitehead 1990; Whitehead & Bannister 1981), softening of the beak tissue (Wakeman 2002), breast blisters (Wakeman 2002), leg abnormalities (metatarsal bones become shortened and the hock joint becomes distorted which may lead to perosis) (Oloyo 1991; Patrick *et al.* 1942; Wakeman 2002; Whitehead 1990; Whitehead & Bannister 1981), and increased mortality (Oloyo 1991; Patrick *et al.* 1942). Some symptoms of biotin deficiency may not be visible externally, such as increased liver and kidney weights, increased lipid deposition within the liver and kidney, raised blood lipid levels, lower blood glucose levels, lower pyruvate carboxylase activity in the liver (Oloyo 1991), and cellular changes such as acanthosis and hyperkeratinisation (Harms & Simpson

1982; Oloyo 1991), abnormal keratinisation and cornification of the epidermis (Buda 2000a; Buda 2000b).

Dobson (1970) observed that turkeys receiving an adequate ration of biotin had soft and pliable footpads, whilst those deficient in biotin had hard and thickened pads, that sometimes also became red or whitish with a 'cooked' appearance before cracks in the footpad appeared.

Platt *et al.* (2001) posed the hypothesis that there was a marginal biotin deficiency in the skin of the turkey footpad and that this deficiency contributes to the development of FPD lesions. This small deficiency may only be problematic in the skin of the footpads. Platt suggested that as footpads are exposed to high mechanical strain from weight bearing, that the skin may become malnourished as a consequence of the increased thickness of the avascular epidermis.

The need for biotin may increase if the diet provided is particularly high in fat or protein. Broilers fed a biotin deficient diet developed lesions that were more severe if they were also fed a high protein diet (Whitehead & Bannister 1981).

3.1.2.3 Different requirements of poults and chicks

Turkeys are particularly at risk from biotin deficiency symptoms, as they have the highest biotin requirement of any bird species investigated so far (Scott 1981; Whitehead 1990). Biotin requirements of turkey poults are higher than those of the chick. Patrick *et al.* (1942), estimated the daily requirement for chicks to be 2

μg/day, and for poults, 5 μg/day during the first four weeks of life. Although the biotin intake is stated as a daily requirement rather than an amount per kilogram as it is usually expressed, it is still evident that poults have a higher need for biotin than chicks. The dietary requirement for optimum performance of both sexes of broiler chicks reared on litter floors is 180μg available biotin/kg of diet (Whitehead 1986), whilst a poult starter ration should be between 250-300 μg/kg (Whitehead 1990; British United Turkeys 2003).

3.1.2.4 Experimental evidence testing recommended biotin levels

Commerically, the following concentrations of biotin are supplied to growing poults in conventional (usually wheat-soya based) pelleted feed: poults aged 0-12 weeks should be fed diets containing 300 μg/kg, and from 12 weeks until kill only 200 μg/kg (British United Turkeys 2003).

Opinions differ in regard to how much biotin is required for optimal turkey growth. When provided with commercial feed only, with no biotin supplementation, poults were observed to exhibit signs of biotin deficiency, specifically lesions of the feet, eyes, vent and beak (Buda 2000a; Patrick *et al.* 1942; Wakeman 2002).

Many claims have been made that commercial rations contain an adequate amount of biotin to prevent FPD in poults. Poults receiving unsupplemented diets did not show biotin deficiency symptoms if the feed (composed mainly of ground yellow corn and soybean meal) contained 250-255 μg biotin (Sullivan & Platter 1969). Robblee & Clandinin (1970) concluded that commercial rations given to turkey poults contained

adequate biotin, as they observed only a very low level of FPD. These authors therefore suggested that FPD occurred as a result of external factors, such as an increased need for biotin as a result of stress or variability of the biotin content of feedstuffs, due to deterioration from storage time or conditions. They did admit that biotin supplementation was effective in reducing, although not completely preventing FPD.

However, others have observed symptoms of biotin deficiency (such as foot pad lesions) when poults were fed commercial rations (Dobson 1970). These symptoms have been reduced in severity or eliminated by varying amounts of biotin supplementation. Richardson & Wilgus (1967) reduced the severity and incidence FPD by adding 250 μg biotin per gallon of water to the drinking water of poults for 7, 21 and 35 days, starting with 9 day old birds. The most effective treatment was a continuation of supplementation until recovery was complete. Since the biotin was added to the water rather than the feed, the results of these experiments are difficult to compare with others.

A supplement of 135 μg/kg of d-biotin to the diet did not reduce the frequency or severity of FPD (Johnson 1967). However, with a supplementation of 250 μg/kg, the poults showed a near total recovery. Older stags (14 weeks) showed no recovery from the condition when given 500 μg/kg orally for 5 weeks. Similarly, older stags aged 22 week old showed no recovery when injected with 1000 μg for the first week, followed by 500 μg weekly. The lesions persisted despite the high levels of biotin provided. A possible explanation for these results is that FPD is preventable in its

early stages by increasing biotin supplementations in young poults, but once the condition has become established, it is harder to reverse the condition.

Jensen *et al.* (1970) fed poults a supplement of 250 μg/kg. The birds were found to have a 0 % incidence of FPD at 2, 4 and 8 weeks. Other poults given a diet containing only 50 μg/kg had over 50% FPD at 4 weeks.

Turkeys were fed either a semi purified ration containing 100 μg/kg, or a commercial ration containing 250 μg/kg, plus supplementation (Marusich *et al.* 1970). Turkeys given the semi-purified ration showed 98 % incidence of FPD when no supplementation was given; this reduced to 7 % with 150 μg/kg supplementation, and dropped to zero at 225 and 300 μg/kg supplementation. Turkeys given the commercial ration showed a 53 % incidence of FPD when no supplementation was given, falling to 5 % when provided with 400 μg/kg supplementation. Turkeys on the commercial ration had more biotin present in the feed than those on the semi-purified ration, and showed less FPD with no supplementation, yet still showed some FPD with a supplement of 400 μg/kg; this could be due to the fact that the birds were heavier and gained weight faster than those on the semi-purified ration.

Misir & Blair (1988) fed 1 day old male Large White turkey poults with a standard commercial diet supplemented with biotin. The diet alone produced 92 % FPD, and when supplemented with 100 μg/kg of d-biotin, the incidence declined to 56 %. Supplementation of 200 and 400 μg/kg totally eliminated FPD symptoms. Growth

rates also increased linearly with biotin supplementation (up to 200 μg/kg), suggesting that growth rates are indicative of biotin status.

Male BIG 6 poults, given supplementations of 220 μg/kg of feed, still developed FPD (Platt *et al.* 2001). By increasing the supplement to 440 μg/kg, the severity of the lesions was reduced (Platt *et al.* 2001). In contrast, Whitehead (1977), claimed that just 300 μg/kg was adequate to prevent FPD in growing turkeys.

Low biotin diets (30 μg/kg) cannot prevent FPD (Whitehead & Bannister 1981). Supplementations of between 2 and 2000 μg/kg have been claimed to prevent FPD in turkeys. Patrick *et al.* (1942) stated that a supplemental ration of 2-5 μg per poult, daily (on top of the commercial ration) given during the first 4 weeks was adequate to prevent FPD. This is difficult to compare with other estimates based on a dietary concentration. Comparisons of figures stating biotin requirements per poult with biotin per kilogram of feed requires a knowledge of how much feed the poult consumes. It was estimated that a poult consumed approximately 1.13 kg (2.5 lb) of feed during the first four weeks of life (Slinger & Pepper 1954), which is equivalent of about 40 g of feed per day per poult. The 40 g of feed consumed must contain 5 μg total biotin if no extra supplementation is given. Feed given to the turkeys must therefore provide 125 μg biotin/kg feed. However, since 1954, feed consumption has increased to 1.58 kg during the first 4 weeks (British United Turkeys 2003). Modern turkey breeds consume an average of 56 g/day of feed during the first 4 weeks of life. Consequently, the feed provided must contain 89 μg biotin/kg feed to satisfy the biotin needs of the poult.

Work by Jensen *et al.* (1970) found no improvement of FPD with injections (150 μg d-biotin) or supplementation (250 μg orally) of biotin when the diet is made up of a high concentration of soybean. Dermatitis occurred despite the increased biotin intake. It may be that these supplementations are not enough to prevent FPD, or that the supplements could not counteract the effects of wet litter, as suggested by Platt *et al.* (2001).

Studies have found that 2000 μg/kg given to stags between 9-20 weeks reduced FPD dramatically (Buda 2000a, Buda 2000b; Wakeman 2000). Previous experiments have found that levels of biotin supplementation a great deal less than 2000 μg/kg can prevent FPD. In the experiments carried out by Buda (2000a), and Wakeman (2000), commercial diets containing an average of 300 μg/kg of biotin were found to result in FPD, so a large supplementation (2000 μg/kg) was provided to remedy this. In Wakeman's study, the biotin treated group had a lower FPD score (1.42) than the control group (1.72) on the Martland scoring system. No other intermediate levels of supplementation were reported to have been tested between 300 and 2000 μg/kg, so a slightly lower level of supplementation may be sufficicent to prevent FPD.

It has been suggested that biotin be used to prevent Bumblefoot in broiler breeders. Bumblefoot is usually bacterial in origin, causing swollen and inflamed foot pads in avian species. Despite the fact that this condition involves a microbial infection, dietary supplementation of 400 μg/kg over a 6 month period did reduce the severity of the condition (Whitehead 1990).

3.1.2.5 Biotin bioavailability in different diets

Biotin is found in a number of food sources with the richest concentrations found in yeasts, nuts and oilseeds. Once consumed, biotin has a propensity to concentrate within the liver, kidney, bone and egg yolk of avians (Wakeman 2002). If the only source of biotin is dietary biotin, then the amount of biotin consumed depends a great deal on the type of diet provided. Commercial starter diets average around 187 μg/kg. The diet for growers is about 196 μg/kg and for breeders is around 147 μg/kg biotin, without supplementation (Richardson & Wilgus 1967). However, the available biotin may vary as a result of storage conditions and over time. The major factor affecting biotin bioavailability is the type of cereal within the feed (Marusich 1970). If the biotin is bound to other molecules, usually a protein via lysine, the links are not able to be broken down in the gut, and are not accessible to the bird (Wakeman 2002). Wheat has a very low biotin availability (17 %) compared with soya (77 %) (Buda 2000b). Corn however is reported to have 95 % available biotin (Clark *et al.* 2002).

Soybean diets produce a high incidence of FPD. This may be due to a low bioavailability of biotin. When 54 % soybean meal replaced casein, gelatin and corn in a purified diet, FPD increased (Jensen *et al.* 1970). Some FPD was observed in poults consuming raw soybean diets, but FPD was increased further when the raw soybean was autoclaved for at least 30 minutes (Jensen *et al.* 1970). Presumably, autoclaving reduces the available biotin. Poults receiving a diet of ground wheat, casein, gelatin and soybean meal exhibited severe symptoms of biotin deficiency, but these were rectified if provided with an extra 100 μg/kg biotin (Sullivan & Platter

1969). The highest incidences (98 %) of FPD were observed in poults fed a diet of corn and soya. Even if fed a supplement of 250 µg/kg biotin, those on the corn-soy diet exhibited 95 % foot pad dermatitis at 10 days of age.

Misir & Blair (1988) provided poults with a standard commercial ration, then replaced the carbohydrate with various test substances. Canola meal (CM) (which has a 65 % biotin bioavailability) alone resulted in 50 % of poults developing FPD, soybean meal (which has a 77 % biotin bioavailability) resulted in a 28 % occurrence level of FPD, yet soyprotein isolate (37 % bioavailability) resulted in a 0 % incidence of FPD. Consumption of various other carbohydrates such as barley (19 % bioavailability), sorghum (30 %), corn (95 %), triticale (16 %) and wheat (17 %) were associated with a 0 % incidence of FPD when given in combination with CM. (The authors did not state if there was any statistical significance between the different diets). Some of these carbohydrates have a low biotin bioavailability, yet there appears to be a synergistic effect created by adding CM, thereby reducing FPD incidence.

3.1.2.6 Dietary factors that may affect biotin bioavailability

Biotin deficiency may be affected by other supplements. In growing chickens, biotin levels are depleted by supplements of high levels of choline and B-vitamin mixtures (Whitehead *et al.* 1976; Whitehead & Randall 1982). The most effective prevention of FPD and hock lesions was a treatment of biotin combined with folic and pantothenic acid. These vitamins were ineffective at preventing FPD without the synergistic effect of biotin (Robblee & Clandinin 1970).

Various drugs and antibiotics have been suggested to affect biotin availability (Waibel *et al.* 1969). High levels of B-vitamin supplementation, zinc bactitracin, and streptomycin were originally thought to interfere with biotin uptake by the birds, but no deficiency symptoms appeared as a result of feeding high levels of these substances (Waibel *et al.* 1969).

Contrary to this, Slinger & Pepper (1954), suggested that the addition of an aureomycin-B_{12} supplement (containing 1.5-2.0 mg vitamin B_{12}, and 1.0-2.0 g aureomycin per lb) to the diet of young turkey poults increased the availability of biotin within the feed, or possibly increased the synthesis of biotin within the avian intestine. This hypothesis was formulated due to the fact that birds provided with the supplementation showed no signs of FPD or other symptoms of biotin deficiency. Of the poults on the control diet, 9 % exhibited FPD after 8 weeks. (No statistical results were reported in this paper.)

Amino acids such as methionine have also been suggested to affect FPD (Chavez & Kratzer 1972; Chavez & Kratzer 1974; Murillo & Jensen 1976). FPD was noted in poults given the basal diet, but if supplemented with methionine the incidence and severity was significantly reduced. Pantothenic acid deficiency also results in abnormalities such as FPD and lesions at the corners of the mouth and eyes in turkey poults (Kratzer & Williams 1948).

Riboflavin was recommended at the level of 2700 μg/kg of feed to ensure normal development during the first 6 weeks of life (Patrick *et al.* 1944). In poults

consuming riboflavin deficient diets, FPD was severe, but even in poults given supplemental riboflavin, there were still a few cases of FPD (Jukes 1935). The lesions described were similar to those seen as a result of biotin deficiency. Dermatitis developed in poults deficient in vitamin G (riboflavin) as early as 8 days old (Lepkovsky & Jukes 1936a; Lepkovsky & Jukes 1936b).

Zinc, manganese and copper are all important in maintaining a healthy epithelium (Ward 2002). A deficiency in these minerals may lead to problems in the development and maintenance of the epithelial structure. The epithelium may be more likely to rupture in deficiency states and lead to FPD lesions. Foot pad lesions were also noted following a zinc deficiency (Whitehead 1990). When provided with zinc as well as an amino acid complex (either lysine or methionine) FPD was significantly ($P<0.05$) reduced in female broilers (Hess *et al.* 2001).

3.1.2.7 Male and female differences and biotin requirements

There seems to be a difference between the skin structure of male and female chickens and turkeys. Female skin contains more fat and less protein and collagen than males. This suggests that female turkey skin may be more likely to tear than male turkey skin, as the protein matrix will be less dense and therefore easier to pull apart (Kamyab 2001). Accordingly, females should be more susceptible to FPD. Halliwell (1975) however, stated that avians of both sexes had little protective fat and connective tissue directly under the metatarsal pad, leaving the footpad prone to mechanical damage.

There is a higher incidence of lesions in males compared with females ($P<0.01$) (Buffington *et al.* 1975; Harms & Simpson 1975; Harms *et al.* 1977; Harms & Simpson 1977; McIlroy *et al.* 1987). Two commercial turkey producers were compared, using the Martland UK scoring system. The mean score for FPD was 2.5 in males, and 2.0 in hens (McLean, unpublished data. Reference from Clark *et al.* 2002). However, other work has reported no significant difference between the prevalence of FPD in males and females (Martland 1984; Ekstrand & Algers 1997).

There is evidence that males require a higher intake of biotin (50 μg/kg) than females to maintain optimum health. The total biotin requirement of male Broad Breasted Bronze turkeys was reported to be 275-325 μg/kg feed for the first 3 weeks of life. Females of the same breed were found to need less, about 50 μg/kg feed less than males (Dobson 1970). Frigg (1984) also found a consistent but small difference between males and females in the amount of biotin required. Males needed an extra 10 μg to maintain the same percentage of growth as females. These results would explain why males are more likely to exhibit signs of deficiency (Harms & Simpson 1975; Harms *et al.* 1977; Harms & Simpson 1977; McIlroy *et al.* 1987).

3.1.3) Pressure and body weight

The most important factor in causing breast lesions (lesions that are often fluid filled, on the breast tissue) is thought to be pressure (Wylie 1999), followed by shearing forces, friction and moisture. These factors may also affect the footpads and cause similar lesions. The skin of the footpads should be slightly more durable than the skin covering the breast.

Commercial turkeys in general have been bred to be larger than the wild type (Wylie 1999) and males are heavier than females. Modern turkeys are less active than traditional turkeys, spending a greater amount of time sitting, thereby increasing pressure on the breast, hock and foot pads (Wylie 1999). Males are more likely ($P<0.01$) to develop FPD lesions (Harms & Simpson 1975; Harms *et al.* 1977; Harms & Simpson 1977; McIlroy *et al.* 1987), which seems logical if pressure is a contributory factor in the cause of FPD, as males are heavier, and so are exerting more pressure on the foot pads.

The heavier the bird, the greater the pressure that will be exerted on the foot pads, as there will be a greater mass per mm^2. Large captive falcons such as the Gyrfalcon or Anatum Peregrine that are kept in wire cages are more susceptible to Bumblefoot infections than smaller captive falcons (Halliwell 1975).

The mass of the bird is affected by diet and sex, and may have an effect on the prevalence of FPD (Harms & Simpson, 1982). As the mass of the bird increases, so does the likelihood of developing FPD. By reducing salt intake, growth rate, and

therefore mass, (as well as litter wetness) was reduced and the incidence of FPD was lower (Harms & Simpson 1982; Murakami *et al.* 2000).

Flocks which did not reach their average target weight were found to have a higher incidence of both hock and breast lesions (McIlroy *et al.* 1987). This goes against previous claims that the heavier the bird, the more likely it is to develop FPD, but it may be that reduced growth rates are associated with the development of FPD. Younger birds are more likely to have softer skin than older birds, meaning that wet or abrasive litter is more able to damage the skin. Contrary to this, Buffington *et al.* (1975) and Martland *et al.* (1984) found no correlation between weight and the incidence or severity of foot and leg abnormalities in Wrolstad White turkeys.

3.2. External factors

Litter quality and type may be important in the development of FPD as they are in contact with the footpad. It is vital therefore to consider these factors. Litter quality may be affected by many variables, for example, moisture levels may be affected by the type of drinkers provided, humidity, season, amount and consistency of faeces, and stocking density. The type of litter provided is also important, as different substrates may absorb varying amounts of liquid, and cause varying amounts of friction on the footpads of the birds.

Lesions develop at points of contact between skin and the ground and it is these weight bearing surfaces that undergo the cellular changes that characterise FPD (Platt

et al. 2003; Whitehead 1990). Therefore Greene *et al.* (1985) concluded that FPD was a contact dermatitis and suggested that poor litter conditions may be responsible.

3.2.1) Wet litter

Foot, breast and hock lesions increased in severity when the litter in the bird's pens was sprayed with water (Martland 1984, Martland 1985). The severity of lesions on the foot pads of individual birds was higher in broilers and turkeys reared in pens containing wet and sticky litter (Harms & Simpson 1975; Harms *et al.* 1977; Harms & Simpson 1977; Martland 1985; Bray 1985; Bray & Lynn 1986; McIlroy *et al.* 1987). These broilers were observed to have severe skin ulceration on the plantar surface of the foot, the caudal aspect of the intertarsal joint and over the sternum. The prevalence of these alterations in skin conditions was significantly different compared with birds raised on dry litter ($P<0.001$) (Martland 1985). Birds sat in the wet litter and developed lesions on the breast as well as on the hocks and feet. By changing the wet litter for dry litter, lesions healed (Martland 1985). In hens housed on a solid floor it was observed that wet litter significantly increased the incidence of FPD (92 % incidence) when compared with dry litter (38 % incidence) (Wang *et al.* 1998).

Martland (1984) observed that deep litter, especially if wet and crusty, increased the likelihood of ulceration occurring on the metatarsal and digital footpads when compared with a thinner layer of litter. Despite the fact that the footpads carry the weight of the turkey, lameness does not often occur as a result. Turkeys reared on wet litter were found, after 20 weeks, to have a larger mean number of lesions, when

compared with those raised on dry litter ($P<0.001$). In turkeys that did have lesions, a larger mean percentage of the foot pad was ulcerated on the feet of birds raised on wet litter when compared with those reared on dry litter ($P<0.001$).

Housing birds on wet litter also increases the chance of faecal adhesion to the feet, which has been suggested to induce FPD (Jensen *et al.* 1970). A mixture of faeces and litter sticks to the foot pad and dries on, becoming extremely solid. This may result in cellular changes within the epidermis simply by having a foreign body stuck to the foot pad and the extra pressure this exerts on the pad, or from the irritants such as uric acid within the faeces.

Wet litter may encourage bacterial growth, which may be a contributory factor of FPD. 'Bumblefoot' lesions are often seen in other avian species, and although FPD is a different condition, there may be similarities between them. 'Bumblefoot' is the name given to any abnormal enlargement of the bird's foot that is associated with bacterial infection (Halliwell 1975). Examination of 'bumblefoot' lesions in birds of prey have identified *Staphylococcus aureus, Escherichia coli, Streptococcus faecalis, Clostridium* spp., *Proteus* spp., and many other opportunistic fungi in association with the lesions (Halliwell 1975; Garner 1989). Hawkey *et al.* (1985) noted a raised white cell count, raised heterophil numbers and fibrinogen levels, which they claimed to be the responses of the blood to the infectious agents. Garner (1989) observed a juvenile male Golden Eagle with bacterial 'bumblefoot' infection associated with a poxvirus infection of the feet.

'Bumblefoot' lesions often occured on one or both feet as a result of mechanical injury to the plantar surfaces of the toes and the metatarsal pads (Hawkey *et al.* 1985). Oedema, hyperaemia, erythaemia, fibrosis, necrosis, and hyperkeratinisation occur as a result of this initial insult (Cooper 1978). Secondary infections (from pathogens such as those identified by Garner (1989) and Halliwell (1975)) often followed the initial lesion. Halliwell (1975) also stated that an adequate supply of vitamins and essential nutrients was vital to ensure a healthy epithelium. An environment free from faeces that may contain pathogens was important to reduce 'bumblefoot' infections and it follows that FPD may be exacerbated by bacterial invasions.

Wet litter was found to contain a higher concentration of nitrogen and have a lower pH than dry litter (Lerner 1996), resulting in higher concentrations of volatile ammonia within the litter. It is the ammonia in the top layer of litter that Lerner claimed causes focal ulcerative dermatitis (FUD) lesions, (such breast lesions have been linked with FPD (Kamyab 2001). Lumb (2002) stated that ammonia builds up in the straw bedding used for ducklings, and as a result the ducklings feet become 'burnt', as early as two weeks of age. Lumb suggested solution is the use of 'Mistral' (produced by Olmix, France), a commercial litter conditioner that can be applied to litter to absorb its own weight in liquid, thereby drying litter and reducing ammonia levels. Contrary to this, Wang *et al.* (1998) found no difference in ammonia content or litter pH between wet and dry litter in laying hens, but this may be because the wetness was created by spraying the litter with water rather than as a result of faeces, which would raise litter ammonia concentrations.

Growth rates were depressed ($P<0.05$) within one week of being in a pen of wet litter. After 9 weeks of being reared on wet litter, the difference between weights of birds on wet and dry litter was highly significant ($P<0.001$). By changing the wet litter for dry litter growth rate recovered (Martland, 1985), and lesions began to heal (Greene *et al.* 1985; Martland 1985). Floor heating dried the litter, improving litter quality, and significantly reducing the incidence of FPD in turkeys at a slaughter age of 8 weeks (Berg & Algers 2004).

Birds reared on wet litter were found, after 20 weeks, to have a significantly lower mean body mass, a larger mean foot area, a larger mean number of lesions, and in those that did have lesions, a larger mean percentage of the foot pad was ulcerated than those raised on dry litter (Martland 1984).

3.2.1.1 Drinker design

Wet litter may be affected by a number of factors such as drinker design. Small cup drinkers reduce litter wetness (compared with 11 other types of commercial drinkers), and thus decreased hock burn which was thought to be exacerbated by sitting in wet litter (Tucker & Walker 1999). Ekstrand and Algers (1997) supported the fact that small cups reduce wetness, finding that turkey flocks with bell drinkers showed a higher prevalence of FPD compared with those flocks with small cup drinkers. Similarly, houses with small cup drinkers had a higher prevalence of FPD than houses with nipple drinkers (Ekstrand *et al.* 1997).

3.2.1.2 Diet and its effects on excreta

Moisture content of the litter will be affected by the consistency of the faeces and this in turn will be affected by the diet (Jensen *et al.* 1970; Ekstrand & Carpenter 1998c). Soybean meal replacements (SBM) contain fat that is problematic to digest (Boling & Firman 1997; Murakami *et al.* 2000). This indigestible fat results in the excretion of watery, sticky faeces. Poor quality fat has a higher percentage of unsaponifiable matter, oxidised fatty acids, and total fatty acid content, also having a reduced percentage of unsaturated fatty acids (Tucker & Walker 1999). All these factors reduce digestibility of the feed and may lead to watery faces and wet, greasy and sticky litter, increasing the probability of the litter adhering to the birds' feet, and increasing the incidence of hock burn in broilers (Tucker & Walker 1999). It has been proposed that it is the undigested part of SBM in the faeces that causes foot pad lesions (Leeson & Summers 1991). Murakami *et al.* (2000) reported that litter wetness score increased significantly when broilers were fed diets of corn and soybean meal, compared with corn-soybean meal and menhaden fishmeal. They suggested that the corn-soybean meal increased wetness because of the higher potassium levels and electrolyte balance in the corn-soybean diet. High protein, low energy diets were found to increase the incidence of wet capped litter and subsequently increase hock burn in broilers (Bray & Lynn 1986). Turkey foot pad dermatitis was also significantly increased with levels of crude protein above 240 g/kg (Berg & Algers 2004). Litter wetness scores also increased significantly as dietary sodium levels increased (Murakami *et al.* 2000). At 0.15 meq/kg of dietary sodium ($NaHco_3$), litter was scored using Murakami's scale (1 being dry, and 4 being wet), and was 1.69, rising to 1.85 when dietary sodium levels rose to 0.25 meq/kg.

High levels of dietary soyabean meal have been linked with an increased prevalence of FPD (Jensen *et al.* 1970).

3.2.1.3 Air temperature and its effect on litter moisture

If air temperature was above 20°C, increasing the moisture content of the litter was found to increase the incidence of FPD (Wang *et al.* 1998). These authors concluded that moisture and temperature are both important factors in the development of FPD in laying hens. If the temperature falls below a set point, the dew-point temperature (determined by the moisture content of the air), condensation occurs on exposed surfaces, including litter (Tucker & Walker 1999). The risk of condensation is increased if the temperature is low, and the relative humidity is high. Houses should be insulated to prevent the inside temperature reaching the dew-point, and hence helping to reduce the incidence of wet litter (Tucker & Walker 1999).

The air temperature will be affected by stocking density, season and the type of circulation system. Alchalabi (2002) stated that poor litter quality and poor ventilation may result in high levels of ammonia, carbon dioxide and hydrogen sulphide. Poor fan ventilation systems (<150 m^3/h/kg) significantly increased the prevalence of turkey foot lesions (Martrenchar *et al.* 2002). By increasing the ventilation rate, litter moisture may be reduced, with a decrease in toxic odours and pH levels. Humidity will also be affected by similar factors; warm air and wet litter will increase humidity levels and wet litter will not dry as readily or quickly.

Lesions of the hock and breast in broilers have been found to be most prevalant during the winter months (October-April in northern latitudes) (McIlroy *et al.* 1987; Bruce *et al.* 1990; Ekstrand & Algers 1997; Ekstrand & Carpenter 1998b; Ekstrand & Carpenter 1998c; Martrenchar *et al.* 2002; Dawkins *et al.* 2004). This was also when litter quality was at its worst. For example, there were significantly ($P<0.01$) more recordings of poor litter quality (i.e. presence of wet and/or sticky litter) during the winter months (McIlroy *et al.* 1987). There is a definite association between FPD and season, mainly related to the effects of relative humidity and outside air quality (Ekstrand & Carpenter 1998c). As relative humidity rose, litter quality decreased; there was a rise in litter caking, litter moisture, litter nitrogen, and litter ammonia (Weaver & Meijerhof 1990). At a relative humidity of 75 %, approximately 54 % of broilers experienced some degree of FPD, whilst at a relative humidity of 45 %, less than 14 % of birds showed evidence of foot pad lesions (Weaver & Meijerhof 1990). Hock lesions were also shown to increase with relative humidity (which increased during the winter months) (McIlroy *et al.* 1987; Tucker & Walker 1999).

3.2.1.4 Stocking density

Foot pad lesions were found to increase as stocking density increased (Hill 1975; Greene *et al.* 1985; Martrenchar *et al.* 1999; Martrenchar 1999; Arnould & Faure 2003). The prevalence of lesions on the breast and hock in broilers also increased when stocking density increased (McIlroy *et al.* 1987; Svedberg 1988; Martrenchar *et al.* 1997). This increase in lesions with increased stocking density may be due to poorer litter quality. There were significantly ($P<0.01$) more cases of poor litter

quality recorded from highly stocked pens (<0.48 sq ft/bird) when compared with low density pens (0.49 or more sq ft/bird). Turkeys had increasing difficulty in maintaining a normal walking gait; the gait score of turkeys increased with stocking density (Martrenchar *et al.* 2002), which may be due to poor litter quality, resulting in a higher incidence of foot pad lesions. Reduced ability to walk is an important welfare and economic factor as birds unable or unwilling to walk due to pain, may suffer hunger, reduced weight gain, thirst and dehydration, and ultimately may die.

3.2.2) Litter type and friction

Flooring types (wire, wood slats, plastic coated wire, and plastic coated metal) have been found to have no effect on the incidence of FPD in broilers (Simpson & Nakaue 1987). This work was supported by Chen *et al.* (1991) who stated that four types of slotted flooring (concrete, wood, fibreglass and PVC) had no significant effect on either the footpad score or prevalence of FPD in market turkeys.

In contrast, Dwarf Leghorn layers were found to have a higher prevalence of dermatitis if reared in wire cages rather than on litter. The use of plastic coated cage floor inserts and wooden perches significantly reduced dermatitis, but was still greater than those reared on litter (Burger & Arscott 1984).

Laying hens reared in wire cages had a significantly ($P<0.05$) increased frequency of foot pad lesions when compared with those reared on litter or free range (Svedberg 1988). Hens in standard wire cages had a 14-15 % incidence FPD, whilst birds reared on litter showed an 8-9 % incidence of FPD (Svedberg 1988). Lesion

incidence increased as feeding area per hen decreased, as wire mesh area increased, as the number of hens per cage increased, as the area per caged hen decreased, and as floor slope increased. (As the floor incline changed from <16 % to >16 %, lesion frequency increased from 11 % to 23 %) (Svedberg 1988).

The type of litter appears to have a marked effect on the incidence of FPD in turkeys (Hester *et al.* 1997), and in 'bumblefoot' infections of birds of prey (Halliwell 1975). Fine and course particleboard residue was compared with hardwood shavings, as the former provides a substrate less able to hold moisture and bacteria. It was found that turkeys reared on fine particleboard has a lower incidence of FPD, breast lesions and foot abnormalities, perhaps due to the fact that there were less jagged edges. However, fine particleboard holds less liquid, and it is difficult to disentangle the relative roles and importance of friction and moisture as causal factors in this experiment.

Ekstrand & Algers (1997) found that poults reared on straw showed a higher prevalence of FPD than those on wood shavings. However, McIlroy *et al.* (1987) and Bruce *et al.* (1990) found no significant difference in the occurrence of hock and breast lesions in broilers reared on straw or wood shavings. Both types of litter were equally as likely to deteriorate in quality and become wet and sticky, and both resulted in the same incidence of lesions.

(4) Discussion

Many factors have been suggested to cause FPD. The two factors that are most likely to affect FPD being the amount of biotin consumed by the birds, and the presence of wet litter. However, the condition is likely to be multifactorial, and other factors such as skin strength, and weight of the bird may also play a part in the development of lesions.

There is evidence to suggest that biotin is involved in skin formation and maintenance in turkeys. Some researchers have claimed that the amount of biotin contained in commercial rations is adequate to prevent FPD in turkeys, whilst others believe that extra supplements are needed. It is likely than extra supplementation is needed, as the amount of biotin in different commercial feeds will differ according to the available biotin in the ingredients of the feed. For example, corn has a very high percentage of available biotin when compared with soya or wheat.

Supplementations of between 250-440 μg/kg have been found to reduce the incidence of FPD. With a biotin intake of this level, the skin of the footpad is adequately supplied with biotin, allowing normal development and maintenance, and the skin of the footpad is protected from tearing and nutritional deficiency lesions.

Biotin requirements may differ between experiments due to other factors not mentioned, such as the bioavailability of other nutrients in the diet, the wetness of the

litter (which itself is affected by various other factors, and is discussed below), how often litter is replaced, ventilation, humidity and temperature within the house.

One factor that has not been given a lot of consideration is the possibility that different breeds may have different biotin needs. Some experiments may claim that turkeys as a species need 250 μg/kg whilst others claim they need 440 μg/kg. This may be due to that fact that the experiments used different breeds, and these different breeds require different amounts of biotin to prevent FPD. For example, modern breeds grow at a faster rate than traditional breeds and so may require more biotin to prevent FPD.

However, despite consuming adequate biotin, wet litter is also known to cause FPD and may overcome the protective effects of having an adequate biotin intake. Poor litter conditions may be the dominant factor in this equation. Since biotin is vital in skin maintenance, a lack of biotin does not allow for normal skin development and repair, meaning that as foot pads become eroded by wet litter and irritants such as ammonia, the damage is not as easily repaired.

Wet litter, from water in the drinkers and water from the bird's faeces is associated with FPD. Many factors affect the moisture within the litter, including the design of the drinker; the diet consumed by the birds (as this influences the consistency of the faeces); air temperature and humidity (season has an effect on air temperature and humidity); high stocking density may lead to a higher rate of litter deterioration; and litter type may affect the amount of moisture that is trapped within the litter.

Wet litter is known to have a higher concentration of nitrogen and a higher pH than dry litter, resulting in higher concentrations of volatile ammonia within the litter (Alchalabi 2002). Ammonia has been proposed as a causative agent of FPD. However, it may be simply the wetness, as continually standing in wet litter will soften the footpads meaning that the skin may be more prone to mechanical damage. Bedsores in man result from the skin remaining moist and subjected to friction for an extended period of time and the same may be true in this situation (Bianchetti *et al.* 1993; Jacquot *et al.* 1999). Skin fragility also increases the likelihood of developing bedsores (Jacquot *et al.* 1999).

Wet litter causes litter and faeces to adhere to the footpads and when dry, this material is extremely hard. Irritation may occur as a result, causing lesions as the result of increasing pressure on certain areas of the pad and resulting in cellular reactions.

Further experimental work should be carried out to determine whether wet litter does increase the prevalence of FPD, as it is difficult to compare studies because management practices vary in different experiments. Also, further work should ascertain the exact amount of biotin required by different breeds, and under different environmental conditions. A hypothesis may be formulated, that biotin needs may be less if the birds are reared under good environmental conditions, since there is less challenge to the foot pads.

Chapter 2

Cellular changes associated with the development of foot pad dermatitis with age in commercial growing turkeys

Introduction

Previous work has examined the progression of FPD externally, indicating that dermatitis begins as scaly skin on the foot pad, developing into cracks, with haemorrhaging and scabs on the metatarsal and digital pads (Whitehead 1977; Greene *et al.* 1985). Previous histopathological studies have mostly examined the cellular changes present after lesions have occurred in both chickens and turkeys (Richardson & Wilgus 1967; Harms & Simpson 1975; Martland 1984; Greene *et al.* 1985; Martland 1985; Gonder & Barnes 1987; Whitehead 1990). One recent study of turkeys (Platt *et al.* 2001) recorded the cellular changes in 6 week old poults, and again at 14 weeks, but no previous study has examined the gradual weekly progression of FPD from the first week of the birds life up to market slaughter age. Also, no previous work has taken samples of externally normal footpads for comparison at gross and microscopic levels.

This experiment examined birds from 1 week of age up to 21 weeks and compared apparently normal foot pads with those with lesions. The aim of this work was to track the development of lesions at a histopathological level in young commercial turkeys to determine the age of onset and the rate of progression of FPD in commercially reared turkeys.

Materials and Methods

Experimental design

The birds were sampled from various units, according to age, on the farms of a commercial turkey producer in the UK. Four birds were sampled from each age group; two apparently normal birds, and two birds with signs of foot pad lesions. Both feet were taken from birds at 1, 2, 3, 4, 5, 6, 7, 8, 10 and 21 weeks of age.

Birds

All birds sampled were Large White Broad Breasted T8 turkeys, and all were male, except birds aged four weeks, which were female, since there were no male birds aged 4 weeks available for sampling. The birds sampled were either found dead or were culled by the farm staff in the poultry houses that morning (no more than 2 hours previously). No obviously diseased animals were used for sampling; culled birds were apparently normal but had been damaged physically. The distribution of injuries was in the order of 70 % broken wings, 20 % head pecked and 10 % vent pecked. All birds used for sampling were therefore injured in some way, whether they were found dead, or culled as a result of serious physical injury. However, none of the injuries were directly related to or should affect foot pad status.

Housing

All birds were fed a commercial pelleted diet *ad libitum* formulated from recommendations by the breeder. The birds were kept in large rectangular barns of several thousand birds housed by age. All birds within one house were hatched at a similar time, within a few days of each other. Birds of different ages were housed on different houses within a relatively close area. All houses were ventilated and temperature-controlled according to recommendations from British United Turkeys (B.U.T.) (2003) for turkeys for the specific age of the birds. The litter in each house was white wood shavings kept at a depth of 10-15 cm.

Sampling technique

Dead birds were collected by the farm staff and R.K.Mayne, and the latter examined the birds on site. The feet from each bird were photographed using a Fuji digital camera and a brief description based on the visual condition of the foot pads was recorded according to an external scoring system. The metatarsal pads were then removed and each foot pad placed into tubes filled with 10 % buffered neutral formalin (BNF). The information for the scoring charts (see Table 2.1.) was obtained from preliminary field examinations, plus samples from turkeys reared in wet dirty conditions to try and induce foot pad lesions. Both scoring charts, external and histopathological, were devised by R.K.Mayne and were used to categorise turkey foot pads throughout the experimental work carried out as part of this PhD research project (see Tables 2.1. and 2.2.). The external scoring scale expanded on

existing scales, providing more detailed categorisation, and allowing for the identification of minor changes on the footpad, which had not previously been possible. The histopathological scale reported here was completely novel, no scoring scale recording cellular changes apparent under the light microscope had been published in the literature. These scoring charts allowed for a more detailed report of the lesion to be recorded.

Table 2.1. External foot scoring system

Score	Description of foot pad
0	No external signs of FPD. Skin of the foot pad and digital pads appears normal, no redness, swelling or necrosis is evident. The skin of the foot pad feels soft to the touch
1	Slight swelling and/or redness of the skin of the foot pad
2	The pad feels harder and denser than a non affected foot. The central part of the pad is raised with swelling and redness and the reticulate scales may be separated. The digital pads may show a similar reaction
3	The central and digital foot pads are enlarged and swollen with red areas, and as the skin has become compacted, the foot pad is harder. The reticulate[2] scales have enlarged and separated, and small black necrotic areas may occur
4	Marked swelling and redness around the margins of lesions occur. Reticulate scales die and turn black, forming scale shaped necrotic areas. The scales around the outside of the black areas may have turned white. The area of necrosis is less than one eighth of the total area of the foot pad
5	Swelling and redness are evident in the central and digital foot pads. The total foot pad size is enlarged. Reticulate scales are pronounced, increased in number and separated from each other. The amount of necrosis extends to a quarter of the foot pad. Small necrotic areas may also appear on the digital pads
6	As Score 5, but with half the foot pad covered by necrotic cells. The digital pads may have up to half of one pad covered with necrotic cells also
7	A foot pad with over half of the foot pad covered in necrotic scales

Table 2.2. Histopathological score for categorising histopathology slides of turkey foot pads affected by FPD

Score	Description	Definition
0	None	No change, sample normal
1	Mild	Hyperkeratosis; 'Horned pegs' of keratin on surface; Epithelial hyperplasia; Compacted keratin on footpad surface
2	Mild	Epidermal acanthosis Increased dermal blood vessel density
3	Mild	Vacuoles in dermis/epidermis Necrotic debris in keratin/epidermis
4	Medium	Presence of heterophils, macrophages and lymphocytes in dermis
5	Medium-Severe	Increased density of heterophils, macrophages and lymphocytes Congested/necrotic blood vessels Necrotic debris of cells in dermis/epidermis
6	Severe	Split epidermis - 1 lesion
7	Severe	Split epidermis - 1+ lesion or 1 very large lesion, more than 1/3 of total sample

[2] The skin of the turkey foot pad is composed of reticulate scales, small, overlapping scales which have a higher keratin content than other areas of the integument.

Sampling preparation and scoring

Samples were transported to the Easter Bush Veterinary Centre (EBVC), Edinburgh. Slices were cut from each sample, processed as standard paraffin wax sections, stained with haematoxylin and eosin and examined by light microscopy. Foot pad sections were scored using histopathological criteria (Table 2.2.).

Results

Normal birds (birds with externally normal foot pads – Table 2.1. and Figure 2.1a.)

In very young birds (up to 4 weeks), the foot pads that appeared normal externally (Figure 2.1a.), were also normal microscopically. At older ages, all foot pads that appeared normal upon first inspection showed evidence of minor cellular changes (classified as a mild lesion, Table 2.1.), suggesting that a lesion was beginning to develop. However, the cellular changes were of a lesser degree than those found in birds with obvious macroscopic lesions. These minor changes (Figure 2.2.) included loosely packed but normal keratin, hyperkeratinisation, keratin formed into 'horned pegs' protruding upwards and outwards from the foot pad, epidermal hyperplasia, epidermal acanthosis, hydropic degeneration in the epidermis and dermis, and an increase in the number of blood vessels in the dermis.

Figure 2.1a. Externally normal foot pads. Bird aged 3 & 5 weeks (External score 0)

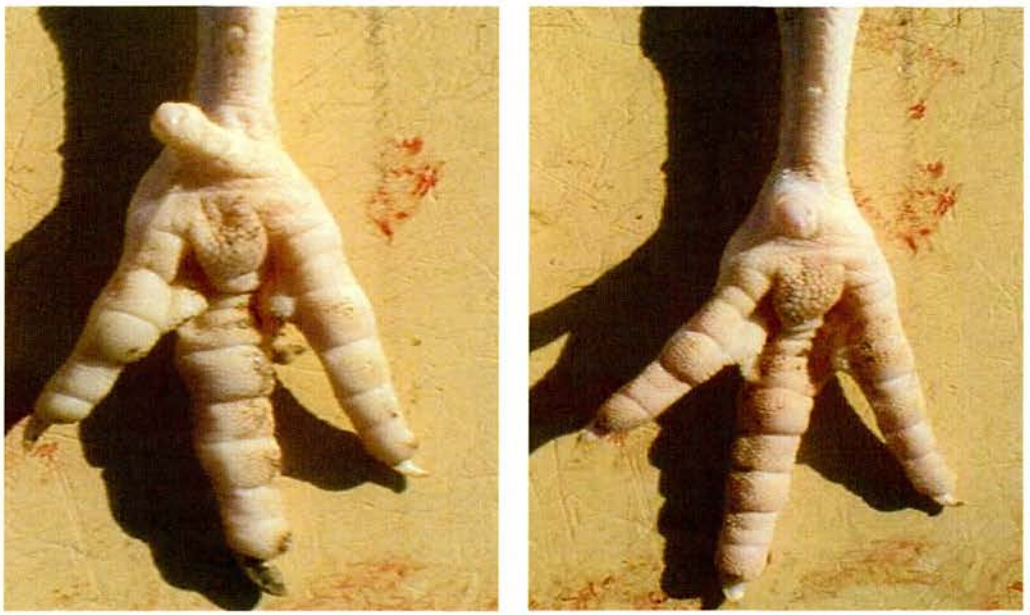
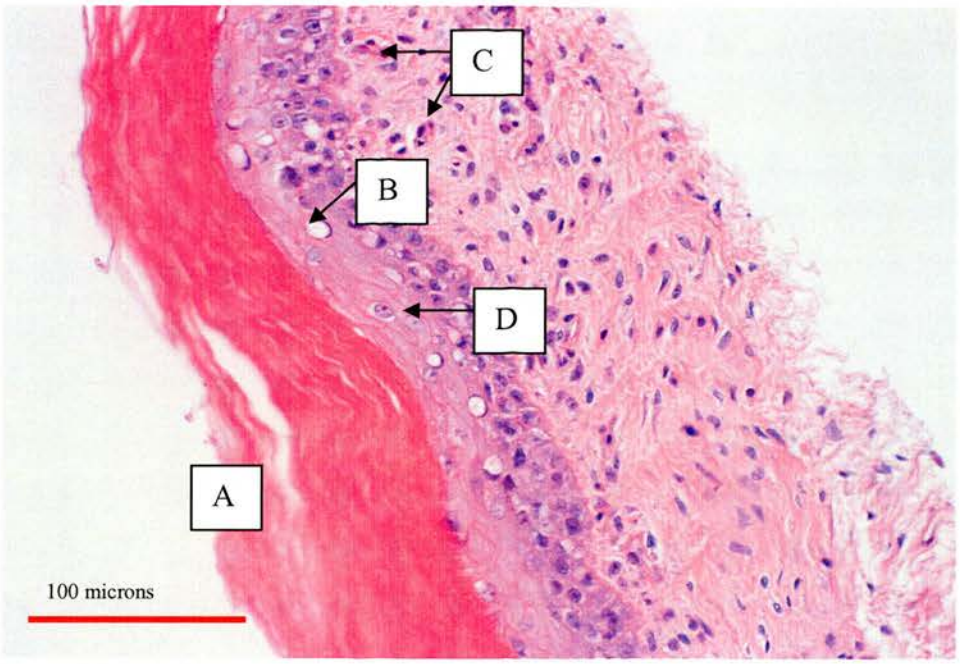


Figure 2.1b. Foot pads affected by FPD. Bird aged 6 & 4 weeks (External score 5 & 6)

Figure 2.2. Minor cellular changes as a result of FPD. Bird aged 3 weeks (Histopathological score 3) (magnification x 10)



KEY

A: Loose and excess keratin

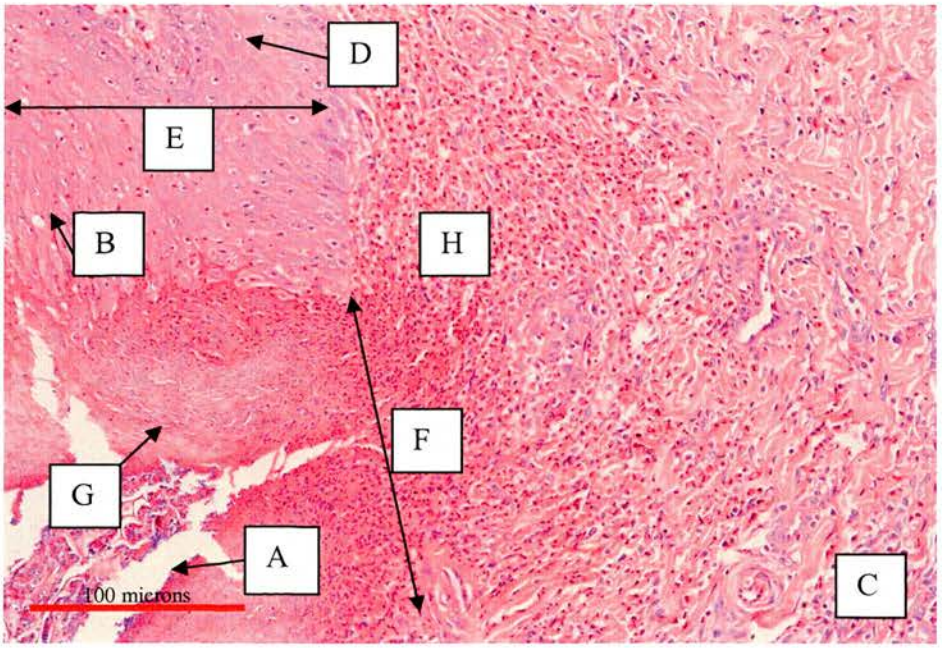
B: Hydropic degeneration

C: Increased blood vessel density in dermis

D: Epidermal acanthosis

Figure 2.3. Major cellular changes as a result of FPD. Bird aged 6 weeks (Histopathological score 7)

(magnification x 10)



KEY

A: Surface keratin has been lost

B: Hydropic degeneration

C: Increased blood vessel density in dermis

D: Epidermal acanthosis

E: Epidermal hyperplasia

F: Ruptured epidermis

G: Scudate (dead and dying cells plus tissue protein exuding through split epidermis)

H: Inflammatory cells in dermis

Affected birds (birds with external signs of lesions on the foot pads - Table 2.1. and Figure 2.1b.)

Birds aged one week exhibited a few minor cellular changes, including hydropic degeneration, acanthosis and an increase in the number of blood vessels in the dermis. The surface keratin showed very minor abnormalities, either loosely packed, in 'horned pegs'[3] (see Fig 2.1b.) or with areas of compressed keratin. The epidermis was normal. There was no evidence of any inflammatory cells within the sample (Figure 2.2.).

At two and three weeks, affected birds exhibited the cellular changes that occurred at one week, but also had deposits of compressed keratin filled with necrotic cells. This compressed keratin either lay in an adherent layer above the normal keratin on the surface or replaced normal keratin completely. A single one week old bird showed evidence of a lesion with a ruptured epidermis and an influx of inflammatory cells from the dermis (Figure 2.3.). In this study a lesion with a ruptured epidermis, either with or without eroded surface keratin, was classified as a fully developed lesion (Table 2.2.).

Birds aged four and five weeks exhibited a greatly increased thickness of the keratin, which was more likely than not to have formed into 'horned pegs'. The epidermis

[3] Pegs of keratin formed from separated reticulate scales, protruding upwards and outwards from the surface keratin of the foot pad

was also thickened and had formed into rete pegs[4]. There was evidence of a few inflammatory cells in the dermis, but not of any great density. These inflammatory cells included large round purple staining cells with granulated nuclei that were interpreted as macrophages. Pink staining cells with polymorphic nuclei were also present and were classified as heterophils. The division of cell types was approximately 70 % heterophils to 30 % macrophages within affected sections of foot pad. Heterophils were found closer to the point of epidermal rupture, whilst macrophages were observed deeper within the dermis.

At six and seven weeks, some birds showed a dense mass of inflammatory cells within the dermis, with an intact epidermis, but in others the epidermis had ruptured (Figure 2.3.). Compressed keratin lay on the surface above the cavity caused by the ruptured epidermis. There was no normal keratin above the lesion, except at the edges of the lesion, where it had not been eroded. The dermis was a dense mass of inflammatory cells (approximately 70 % heterophils and 30 % macrophages), associated with a marked increase in blood vessels, some of which were congested. These inflammatory cells were occupying the site of the ruptured epidermis. Some cells were located between the epidermis and the dead compressed keratin on the surface of the sample.

After eight to ten weeks, the lesions had become more severe, in some cases the compressed keratin on the surface of the lesion had been totally eroded (since the epidermis was no longer present), exposing a dermis completely infiltrated by

[4] Peg shaped areas of thickened epidermis which protrude downwards into the dermis

inflammatory cells, that were mostly heterophils (approximately 70 %). The dermis also showed evidence of apoptotic nuclei, and even some macrophages in deeper layers. These birds frequently had more than one lesion per foot pad, with as many as five lesions recorded in a single sample.

At twenty-one weeks of age, the birds showed more necrosis of cells within the epidermis, resulting in a larger lesion, but they also showed an increase in the amount of compressed keratin (full of necrotic cells) on the foot pad surface. The surface of the foot pad often appeared black due to necrotic cells within the foot pad. There was also an increased number of heterophils and macrophages within the dermis. These older birds showed evidence of chronic inflammation, rather than the acute inflammation in the younger birds. The characteristics of chronic inflammation were epidermal thickening due to acanthosis and hyperkeratosis (both of which were observed in younger birds, but were notably more pronounced at 21 weeks than in younger birds), the upper and mid dermis were infiltrated by inflammatory cells (80 % macrophages, 20 % lymphocytes) which were concentrated around the blood vessels. The epidermis sometimes reformed over the site of rupture, yet the dermis was still full of inflammatory cells.

Discussion

This experiment has shown that cellular changes occur in the foot pad integument, and progress relatively quickly, from a very young age. Although this was a small scale experiment, with only 40 birds sampled, this work demonstrates the development and time course of FPD in young growing turkeys, which has not been fully documented before. As the birds aged, the severity, size and number of lesions in affected foot pads increased.

Fully developed lesions (i.e. the epidermis has split) were observed when the turkeys were just three weeks old, and they became increasingly more common with time. By 6 weeks of age, almost every bird with external evidence of a lesion had histopathological confirmation of a split epidermis. Histopathological lesions did not change a great deal after 6 weeks of age. The surface keratin became more eroded, and an increase in cell necrosis in the dermis and epidermis occurred. In older birds, acute inflammation became chronic, as the skin of the foot pad attempted to compensate for continued insult. In some cases the keratin and epidermis appeared to have repaired, but a large number of inflammatory cells (heterophils and macrophages) remained in the dermis. The surface of lesions were black as a result of cellular necrosis.

Externally, the lesions began as small hard discolourations of the skin progressing into fissures, haemorrhaging and scabs on the metatarsal and digital pads along with acute inflammation and necrosis of the epidermis in mature lesions as has been

reported by Greene *et al.* (1985) and Whitehead (1977). At a cellular level hyperkeratosis of the surface keratin and epithelial hyperplasia were often observed and these changes are consistent with previous observations (Whitehead 1990). Plant material and bacteria were usually present on the surface, but not in deeper layers, suggesting that FPD is not a response to bacterial invasion. The number of lymphocytes and granulocytes, as well as the number of lymphoid follicles in the dermis adjacent to the lesions increased.

Mild lesions were accompanied by an infiltration of heterophils into the stratum germinativum and defects in keratin formation as observed by Martland (1984). In more severe lesions, heterophils filtered into the dermis, sub-epidermis and epidermis. There were also necrotic cells in the keratin layer. Intra-cytoplasmic vacuoles were observed in the epidermal cells. At the centre of the lesion, the epidermis and keratin were often completely destroyed, being replaced by a mass of inflammatory cells, confirming earlier observations (Greene *et al.* 1985). Dermal capillaries were congested and dilated, and there was epidermal hyperplasia at the edges of the lesion. The keratin layer had more obvious defects in formation and often formed 'horned pegs' as noted by Martland (1984), Greene *et al.* (1985), and Whitehead (1990). As the birds aged, fewer superficial lesions were observed, whilst the number of ulcerative lesions that spread into the dermis increased as has been noted previously in comparison of 6 and 14 week turkeys (Platt *et al.* 2001).

Foot pad lesions have also been studied in broiler chickens but, as yet, no-one is certain of the cause in chickens or turkeys. Factors in the external environment such

as the condition of the litter (Lumb 2002; Harms *et al.* 1977; Harms & Simpson, 1977; Martland 1984; McIlroy *et al.* 1987) and components of the diet (Harms *et al.* 1977; Harms & Simpson 1977; Harms & Simpson 1975; Platt *et al.* 2001; Whitehead & Bannister 1981) have been associated with the development of FPD. It is also possible that FPD is caused by single or combinations of internal factors such as the structure of the skin, the sex of the birds, or the genetic potential for rapid growth and high bodyweight (as increased bodyweight increases the pressure exerted on the footpad). Whilst many individual factors have been reported to affect the prevalence of FPD, the condition is likely to be multifactorial in origin. Further experimental work is required to determine exactly what causes FPD, and how the pathological changes develop. Previous experimental work has demonstrated that litter quality and dietary biotin levels had an effect on the development of FPD lesions. As a result of this previous information, work as part of this PhD has included experimentation to determine the effects of litter quality and type and the effects of dietary biotin on the development of FPD. An examination of inflammatory cells present within affected and non affected turkey blood was also carried out in order to examine cellular changes as a result of FPD lesions.

In conclusion it has been shown that there was cellular evidence of an inflammatory response associated with subsequent FPD in commercial turkeys at an earlier age than previously reported and the changes apparently proceed rapidly to mature lesions in less than 3 weeks. However, since this experiment was of a small scale, sampling 40 birds only, further work may be needed to confirm conclusions as to the pathogenesis of this condition. Despite this small scale, this work demonstrates how

the pathological changes develop in young turkey poults and the results are important in understanding the development of FPD as well as its fundamental cause.

Chapter 3

Effects of litter type and quality on the development of turkey foot pad dermatitis

Introduction

Good litter management is vital to maintain a good quality of animal welfare, as well as having a possible effect on FPD and other dermatological problems such as sternal bursitis (breast blisters) and focal ulcerative dermatitis (breast buttons). Dietary factors may also affect the amount of liquid consumed, and therefore excreted by the birds into the litter (Bray & Lynn 1986), and the absorbency of the litter is therefore important. Litter wetness is clearly associated with an increasing prevalence of FPD in turkeys and broilers (see Chapter 1) and it is essential to control litter conditions to ensure that litter does not become too wet. Litter greasiness may also affect FPD (Bray 1985). Diets containing indigestible fats resulted in fatty and greasy excreta from the birds, which caused litter to become capped and less friable (Bray 1985). Litter pH also increased from 5.2 to 8.2, suggesting that the hock burns described were not as a result of 'acid burns' as had been suggested previously (Bray 1985).

The absorbency of the litter, the thickness it is spread on the floor of the house, and the density of birds within the house will all affect the wetness of the litter. The ventilation system within the house is also an important variable in controlling litter wetness. Outside temperature may be an important factor in the development of FPD, as during colder months, ventilation rates are often reduced to decrease heat loss. As a result of reduced air flow, litter may not dry out as rapidly (Tucker & Walker 1999). One way to reduce litter wetness has been found to be effective, and this is under floor heating (Dobrzanski & Bialas 1993). Floor heating was found to reduce the amount of heat lost through the ceiling, walls and ventilators, that

occurred with the use of conventional warm air or radiator systems in bird houses. (Dobrzanski & Bialas 1993).

Stocking density has an effect on FPD (Martrenchar 1999). The obvious effect of increasing stocking density is that there will be a greater volume of excreted matter per unit area, reducing the litter quality. Martrenchar (1999) and Martrenchar *et al.* (1999) reported that birds at the higher stocking density showed a significantly higher incidence of hip and foot lesions, a decrease in activity levels and lower body weights. This decrease in gait scores may be due to reduced activity levels, or it may be due to increased frequency and severity of FPD lesions (Martrenchar *et al.* 1999). These results suggest that increased bodyweight was not responsible for inducing FPD lesions in this case.

Pine wood shavings (shavings are thinner and smaller slices of wood than wood chips, but larger than sawdust, which is almost like powder) are the most common bedding material used for poultry in the UK and North America (Malone 1992). Shavings may exhibit increased moisture and caking when compared with sawdust. Many different processed wood products have been used for bedding materials, including woodchips, shredded wood pallets and wood fibre pellets. Woodchips, that is small, flat pieces of wood, from pine or hardwood trees were found to have a higher initial moisture level, but did not become as compressed or degraded as rapidly as shavings or sawdust. Sawdust is more powdery than woodchip, as the pieces of wood are far smaller, so this bedding is more easily compacted. Shredded wood pallets were found to have a low moisture level, and an absorbency

comparable to conventional wood bedding (White & McLeod 1989). When turkeys were reared on wood fibre pellets, the result was less litter caking than shavings (Nakaue *et al.* 1985). Of all the plant materials that have been examined for bedding materials worldwide, rice hulls is the most popular, and is comparable with wood shavings (Malone 1992).

Recycled paper chips were tested and were found to be a suitable litter material for broilers (Lien *et al.* 1992). The incidence of breast blisters and leg abnormalities was lower in broilers reared on paper chips, despite an increase in moisture and caking compared with wood shavings. This may be due to the less abrasive nature of paper chips and their flattened structure when compared with pine shavings (Lien *et al.* 1992). The moisture content and caking levels within paper litters (both processed and shredded newspaper) was found to be higher than that of hardwood sawdust. Broilers on processed cardboard showed a greater incidence of breast blisters when compared with other litters; this may be due to the glues used in cardboard production, combined with the greater litter moisture which was associated with litter caking and increased breast blisters (Malone *et al.* 1982; Lien *et al.* 1992). Malone *et al.* (1982) concluded that cardboard was not a suitable litter material for broilers due to the increased caking, litter moisture and breast blisters. It has been suggested that since recycled paper products result in high moisture and caking levels, they may be most useful as a base layer or a top layer of litter in combination with other wood based litters to absorb moisture (Malone 1982). The absorbent nature of wood based litters combined with the less abrasive nature of paper products may be a good

solution for increasing the absorption of poultry bedding whilst reducing abrasiveness.

Using waste products for bedding may be another possibility. However, the reuse of polystyrene and plastic as bedding materials, although having excellent characteristics, presents a disposal problem after use as these products are not biodegradable (Malone 1992). These products do not absorb water, but liquid runs off them, allowing for the top layer of bedding to remain dry. They are also composed of smooth materials, reducing friction on the birds skin and foot pads. Another possibility for using waste products as bedding is the use of composted municipal waste, which when tested showed characteristics similar to wood shavings (Malone *et al.* 1983).

Overall, there are a number of different types of litter materials that have been tested for use as poultry bedding. Advantages and disadvantages of different materials must be balanced to find the most suitable litter for the environment.

The following three experiments aimed to examine more closely the relationship between external environment, notably litter type and quality, and foot pad dermatitis. The first two experiments examined the effects of both wet and dry woodchip, whilst the third experiment tested the effects of different litter substrates, both wet and dry on FPD. Since litter wetness has been shown to affect FPD, this set of experiments was conducted to define any differences in foot pad lesions as a result of birds being housed on wet litter, compared with birds housed on dry litter.

Poor litter quality has also been suggested to be important in the development of FPD, so wet dirty woodchip litter was used as a test substrate in the first 2 experiments and foot pad scores were compared with birds housed on wet clean woodchip litter. In the third experiment the litter type was examined. Different litter types will have different absorbencies and may affect the development of FPD if wetness is a factor. It is possible that litter derived from wood products may be causing irritation of the foot pad skin as a result of the wetness leaching an unidentified irritant substance from the wood. These experiments aimed to test the hypotheses that increased litter wetness, poor litter quality and litter type increase the prevalence turkey foot pad dermatitis.

Materials and Methods

Birds

All experiments began with newly hatched female T8 Large White Broad Breasted turkeys from Bernard Matthews' farms, Norfolk, (breeding stock originally supplied by B.U.T.) fed a standard commercial pellet ration suitable for birds aged 0-5 weeks of age. Experiment 1 used 96 birds housed across 12 pens. Experiment 2 used 80 birds housed in 12 pens, and experiment 3 utilised 192 birds in 32 pens.

Housing

Each pen contained a hanging bell drinker, a round feed dish placed on the pen floor for the first 5 days, after which the food hopper was wall mounted (except in experiment 2 where a suspended tubular feeder was used) containing the relevant diet. The pens also contained a suspended heat lamp for the first 7 days. Pens containing wet litter were lined with thick plastic and taped to the sides of the pen to contain moisture and prevent contaminating pens containing dry litter. The air temperature was 28°C throughout each experiment. Low stocking density was maintained throughout all experiments to optimise litter scores. The larger pens used in experiment 2 increased the unit area per bird.

Experiment 1: Birds were housed from day old in 12 pens, each 1 m by 1 m. There were 8 birds per pen. Each treatment was replicated 3 times. Treatments were allocated at random across the whole experiment.

Experiment 2: All birds were housed in a single large pen littered with standard woodchip for 7 days. After 8 days, 6 birds were transferred to one of 12 pens, measuring 3 m by 2 m. Treatments were allocated at random across the whole experiment. Each treatment was replicated 6 times.

Experiment 3: Birds were housed from day old in 32 pens, each 1 m by 1 m. There were 6 birds per pen. Treatments were allocated at random within 4 experimental blocks. Each treatment was replicated 4 times.

Lighting

Light conditions were maintained at a low intensity of 20 lux throughout the experiment to keep cannibalism or any damaging pecking between birds to a minimum. The photoperiod schedule was 16 hours of light with 8 hours of darkness.

Experimental treatment conditions

Experiments 1 & 2: Birds were housed on clean dry woodchip for the first 28 days. After this period they were housed on their treatment litter for 8 days. In experiment 1, the treatments were dry clean woodchip, wet clean woodchip, wet dirty woodchip, and a dry floor with no litter (which was scraped of faeces and spilled water daily). In experiment 2, the treatment litters were either wet clean woodchip, or dry clean woodchip.

Experiment 3: Birds were housed on clean dry litter of the type that they would be housed on during the experimental period for the first 28 days. After 28 days, birds were housed on one of 4 different litter treatments, woodchip, recycled cardboard chips (manufactured by BedXcel, Ayrshire, UK), recycled paper shreds (manufactured by Envirobed, Preston, UK), and long barley straw, all of which were used both wet and dry, totalling 8 different treatments.

Litter quality

Litter was monitored using a scale adapted from Tucker and Walker (1999) and shown in Table 3.1. In pens with clean litter treatments, dirty litter was replaced with clean throughout the experiment, even in wet pens, to maintain constant experimental conditions. In pens containing dirty litter, litter was collected from the first 28 days of the experiment and applied during the experimental period. Pens containing dry litter were maintained at a score 1 on this scale, by removing wet litter from these pens and replacing it with dry woodchip as required. Pens containing litter that was meant to be wet, were maintained at a score 4-5 by adding water daily. Water was distributed evenly across all litter within the pen using a 5 litre bucket. In the small pens (1 m x 1 m) used in experiments 1 and 3, 1 bucket of water (approximately 5 l) was applied daily to wet pens to maintain a score of 4-5 on the scoring chart. In the larger pens (3 m x 2 m) in experiment 2, 2 buckets of water (approximately 10 l) were applied daily to maintain a score 4 on the scoring chart.

Table 3.1. Scoring system for litter quality (Tucker & Walker 1999)

Score	Description of litter condition
1	Dry, as at day old
2	Slightly damp/tacky
3	Damp/tacky. Sticks to bird's feet but some dry areas still accessible
4	Most areas wet/sticky greasy (bird's feathers likely to be soiled)
5	Soggy, squelchy or very wet/greasy. Leaves durable imprint when compressed or very slippery (could be on top of a cap)

Litter sampling

Litter samples were taken from experiments 2 and 3. Litter moisture was determined by taking 4 samples from each pen, one from each corner, mixing the samples, and subsampling about 100 g into weighed plastic trays. These were reweighed to determine the quantity of fresh litter. The samples were dried in an oven at 60°C for 1 week, reweighed and the percentage moisture calculated from the loss in weight.

Bird culling

Birds were injected intravenously into the brachial wing vein with approximately 2 ml of sodium pentobarbitone to ensure humane culling.

Foot pad data recording

After 28 days of rearing on clean dry woodchip (day 0 of the experimental period), the birds were weighed, external foot pads examined and assigned a foot pad score

using the external scoring system (described in Chapter 2, Table 2.1.) before the treatment litter was allocated to each pen. At the start of the experimental period 1 bird per pen was selected at random on the basis of wing band number and culled for external and histopathology scoring of the foot pad.

Experiment 1: 1 bird per pen was culled for external and histopathology sampling during the experimental period at days 2, 4, 6 and 8.

Experiment 2: After 6 days of the experimental period, all remaining birds (5 birds per pen) were culled for external and histopathology samples. Since the previous experiments showed the development of lesion severity with time, this experiment took foot pad samples only at the start and finish, that is, days 0 and 6. This sampling timetable allowed for a smaller number of birds to be involved in the experiment, but was sufficiently powerful to detect a 1 point difference on the histopathological scale. A power calculation was carried out to ensure that enough birds were used to detect a significant difference. All birds were weighed after death and before foot pads were removed for histopathological sampling.

Experiment 3: 1 bird per pen was culled for external and histopathology sampling during the experimental period at days 2, 4 and 6.

Foot pad sample preparation

After culling, the right footpad from each bird was scored externally as described in Chapter 2 (Table 2.1.). The skin of the footpad was removed and stored in 10 %

BNF. Sections were prepared and processed using standard protocols for section processing. Slices of the affected area of the foot were cut using a scalpel, placed in labelled tissue cassettes and stored in 10 % BNF again to preserve them until the tissue was embedded in wax. Wax embedded tissues were cut into 2-5 micron sections using a microtome. The sectioned tissues were mounted onto microscope slides and stained using haemotoxylin and eosin (H & E) stain. A cover slip was adhered with Depex mounting material over the top of the tissue. Sections were examined under the light microscope and categorised using the histopathological scale described in Chapter 2 (Table 2.2.).

Ammonia measurements

Atmospheric ammonia measurements were taken during experiment 3, using a handheld Draegar meter tube (Ammonia 2/a) used with a Draeger pump (Draeger CMS, Lubeck, Germany). Ammonia levels were recorded from each pen at day 0 and 6 of the experimental period. The measurements taken at day 0 were carried out before litter was wetted, and were essentially measurements of ammonia levels from dry litter. The glass Draeger tube was broken at both ends and inserted into the Draeger meter. The meter was then held about 2-3 cm from the litter in the middle of the pen in front of the wall mounted feeder. The Draeger meter was pumped by hand to suck air through the tube for 1 min. The extent of discolouration within the Draeger tube was then read off the tube and recorded. The pump was flushed with air after each measurement.

Statistical analyses

Data were analysed using a general split plot ANOVA within the statistical program GenStat (GenStat Release 8.1. Lawes Agricultural Trust, Rothamsted Experimental Station). The statistical model for both external and histopathological foot scores included effects for block (experiments 2 and 3) and treatment with age (days) as a split-plot effect. All residual errors were normally distributed which allowed for analysis without the transformation of data. Ammonia concentrations were transformed using logarithms of the raw data to normalise residual errors.

Experiment 1: Effects of wet and dirty litter on turkey foot pad dermatitis

Results

External foot scores

External scores at the start of the experiment showed that birds on all litter treatments had no external signs of lesions (Table 3.2.). Throughout the experiment, birds housed on clean dry litter showed the mildest external signs of lesions after 8 days. Foot scores were 0.67 after 2 days, and stayed at the same score until day 8. In all other litter treatments, the mean external foot scores rose steadily throughout the experiment with time. After 8 days, the highest mean external foot score was recorded in birds housed on wet dirty litter (3.00), although the foot scores from birds housed on wet clean litter were only slightly lower (2.33). There was no statistically significant effect of litter ($P > 0.477$), or litter and day ($P > 0.459$) on external foot scores. However, there was a statistically significant effect of sample day on external foot score ($P < 0.001$). The SED (Standard Error of the Difference) between external scores at different sample days was 0.33, between scores from birds on different litter types was 0.70, and for sample day and litter, the SED was 0.92.

Table 3.2. Mean external scores of turkey foot pad severity as a result of housing on different litter types over 8 days (SED 0.92)

Litter	Day 0	Day 2	Day 4	Day 6	Day 8
Clean dry woodchip	0.00	0.67	0.67	0.67	0.67
Clean wet woodchip	0.00	1.00	2.00	2.33	2.33
Wet dirty woodchip	0.00	1.00	1.67	2.00	3.00
No litter	0.00	1.33	1.33	2.00	1.33

Histopathological foot scores

Histopathological results (Table 3.3.) showed that by day 8 of the experimental period, birds housed on wet clean litter (5.67) showed the most severe foot pad lesions, as categorised by the histopathological scale (Table 3.3.). Birds on dry clean litter showed the mildest scores, the mean foot score at day 8 was 1.67. Birds housed on either no litter, or on wet dirty litter exhibited similar foot pad scores of 4.00 and 3.33 respectively at day 8. From day 0 to 8, the histopathological foot scores of birds generally increased with time. There was a statistically significant effect of sampling day ($P < 0.022$). There was no significant effect of different litter treatments ($P < 0.13$), or treatments and day ($P < 0.743$). The SED between external scores at different sample days was 0.90, between scores from birds on different litter types was 0.72, and for sample day and litter, the SED was 1.76.

Table 3.3. Mean histopathological scores of turkey foot pad severity as a result of housing on different litter types over 8 days (SED 1.76)

Litter	Day 0	Day 2	Day 4	Day 6	Day 8
Clean dry woodchip	2.00	1.67	2.00	3.67	1.67
Clean wet woodchip	1.67	2.00	4.33	5.67	5.67
Wet dirty woodchip	1.33	3.00	4.67	3.00	3.33
No litter	1.33	3.67	6.00	4.67	4.00

Discussion

These data suggest that wet litter alone may be the most important factor in the development of foot pad lesions in young turkey poults. Although there were no statistically significant differences between different litter treatments and foot scores, the general trend did appear to show that birds housed on wet litter, either clean or dirty, resulted in higher foot pad scores than birds housed on clean dry litter. It may be that this experiment was too small to detect a significant difference between foot scores. An important factor to note is that there were histopathological changes that occurred at a cellular and tissue level that were not detectable on the external footpad.

Throughout the experiment there were wide variations in the severity of both external and histopathological lesions within a particular treatment group, probably due to individual differences. However, it was still possible to observe general trends within each group.

Birds housed on clean dry litter showed minimal signs of foot pad lesions, both externally and histopathologically. Birds housed on wet clean litter showed the most severe histopathological lesions. Birds housed on no litter or wet dirty litter exhibited a similar severity of histopathologically lesions. However, the external scores showed little difference between the birds housed on wet clean litter and wet dirty litter. It may be that the faeces on wet dirty litter mask an irritant being released from the litter. Alternatively, it may be that it is simply wet litter, regardless of whether it is clean or dirty, that induces lesions.

Histological foot scores became significantly worse as time progressed, with scores becoming gradually more severe from day 0 to 8. Histological scores also became numerically more severe in birds housed on wet when compared with dry litter.

The results of experiment 1 suggested that wet litter alone was the primary cause of FPD in turkeys and experiment 2 was designed to test this hypothesis.

Experiment 2: Effects of litter wetness on the development of turkey foot pad dermatitis

Results

External foot scores

There was a highly significant effect ($P < 0.001$) of the litter treatments and day on external foot scores. The interaction of both day x wet/dry treatments was also highly significant ($P < 0.001$), that is, there was a significant difference between external foot scores from birds on wet or dry litters at different days. The mean external foot score (Table 3.4.) from birds on wet litter (6.33) was a great deal higher than scores for birds housed on dry litter (1.83) at day 6. The SED between days was 0.32, between treatments it was 0.36, and for day x treatment the SED was 0.53.

Table 3.4. Mean external foot scores from birds housed on dry or wet litter with time

Litter Treatment	Day 0	Day 6
Wet clean woodchip	2.50	6.33
Dry clean woodchip	2.50	1.83

Histopathological foot scores

There was a highly significant difference between foot scores from birds housed on wet or dry litter ($P < 0.001$). There was also a highly significant difference ($P < 0.001$) between foot scores from birds at different days and from wet/dry treatments x day.

The mean histopathological foot score (Table 3.5.) from birds on wet litter (6.53) was higher than the mean score for birds housed on dry litter (2.53) at day 6. The SED between days 0 and 6 was 0.29, between treatments it was 0.32, and the SED for day x treatment was 0.41.

Table 3.5. Mean histopathological foot scores from birds housed on wet or dry litter with time

Litter Treatment	Day 0	Day 6
Wet clean woodchip	2.50	6.53
Dry clean woodchip	2.50	2.53

Litter moisture

The percentage litter moisture (Table 3.6.) calculated from litter samples showed that the percentage moisture from pens with dry litter was between 9.2 and 19.2 %, whilst the percentage litter moisture from pens with wet litter was between 69.0 and 77.1 %.

All pens at day 0 contained dry litter. There was a highly significant effect of treatment, day, and treatment x day ($P < 0.001$). The SED between days 0 and 6 was 1.41, between wet/dry treatments it was 1.71, and for day x wet/dry treatment it was 2.213.

Table 3.6. Mean litter moisture from pens at day 0 and 6 of the experimental period

Litter Treatment	Day 0	Day 6
Wet clean woodchip	12.94	73.85
Dry clean woodchip	14.16	12.16

Discussion

The amount of moisture within litter from wet pens was within a fairly narrow range (69.0 to 77.1 %). This suggests that the amount of water applied to each pen maintained a similar level of moisture within each pen. The level of wetness was also maintained at a score 5 on Tucker & Walker's scale (1999).

Analysis of external foot scores showed that there was a highly significant difference between external foot scores from birds on wet clean woodchip, and dry clean woodchip. Mean external foot scores at day 6 were 6.33 from wet clean woodchip, and 1.83 from dry clean woodchip. The external foot scores from birds housed on dry clean woodchip showed a slight anomaly; the mean foot score decreased from day 0 to 6. This difference was probably due to the small sample at day 0. The external foot scores at the start of the experiment were calculated from the 12 birds killed for histopathology, whilst the external scores for day 6 were calculated from the remaining 5 birds within each pen, totalling 60 birds.

These data suggest that wet litter had a highly significant effect on external foot scores in this experiment, and a produced a large increase in external foot scores over a relatively short time period.

Histopathological foot scores showed a highly significant difference between birds on wet clean woodchip (6.53), and birds housed on dry clean woodchip (2.53). The histopathological foot scores from birds on dry woodchip were very similar at day 0 (2.50) and day 6 (2.53), unlike the external foot scores at these times.

Histopathological foot scores provide more detailed data about the cellular changes occurring within the foot pad than external foot scores, so it is interesting to note that histopathological foot scores were very similar at day 0 and 6, reflecting the fact that litter moisture (quality) was similar throughout the experiment.

From these data it can be concluded that wet litter had a significant effect on histopathological foot scores in this experiment. Foot pad lesions developed very rapidly within the 6 days of this trial, with histopathological and external foot scores increasing by about 4 points from day 0 to 6.

Experiment 3: Effects of different litter types and wetness on the development of turkey foot pad dermatitis

The results of the previous experiment (experiment 2) suggested that wet litter is an important factor in the development of foot pad lesions and we posed the hypothesis that an irritant was released from wood chip after it became wet, which was causing FPD lesions to develop. This experiment aimed to test different types of commercially available bedding to see if there was any difference in the development of lesions on different treatments when they were either wet or dry.

Results

External foot scores

There was a highly significant effect of day on external foot scores ($P < 0.001$), i.e. external foot scores worsened as the experiment progressed. The effect of day x wet /dry treatment was also highly significant ($P < 0.001$). There was also a significant effect of day x litter at the 5 % level. SED are presented in Table 3.7.

Table 3.7. SED between external foot scores from different factors

Experimental factors	SED
Mean scores on different litters	0.52
Mean scores from wet/dry treatments	0.37
Mean scores from day x litter	0.67
Mean scores from day x wet/dry treatments	0.47
Mean scores from litter x wet/dry treatments	0.73
Mean scores from day x litter x wet/dry treatments	0.95

The results in Table 3.8. show that on day 0 recycled cardboard, recycled paper and woodchip had similar mean scores whereas the score for straw was significantly higher than for woodchip ($P < 0.001$). This pattern of litter effects was seen in both wet and dry pens on days 2 and 4. By day 6 all mean scores were similar apart from those on dry recycled paper and woodchip. Generally there was an increase in mean score from day 0 to 6 in all birds housed on all litters. Foot scores from birds housed on straw began the experimental period with higher foot scores than on other litters.

Table 3.8. Mean external foot scores from birds housed on different litter types, both wet and dry, with time

Litter		Day 0	Day 2	Day 4	Day 6
Recycled cardboard	Dry	2.38	2.75	3.50	5.00
	Wet	-	3.75	4.00	5.50
Recycled paper	Dry	1.75	2.75	3.25	1.75
	Wet	-	3.00	4.50	5.75
Straw	Dry	4.75	4.50	5.50	4.75
	Wet	-	4.75	6.00	5.25
Woodchip	Dry	2.12	2.50	3.50	1.00
	Wet	-	3.25	4.00	4.75

Histological foot scores

There was a highly significant ($P < 0.001$) effect of day on histological foot scores i.e. histological foot scores worsened as the experiment progressed. There was a

significant effect ($P < 0.001$) of day x wet/dry litter treatments on histological foot scores at the 5 % level. The SED between mean histological foot scores as a result of different factors is presented in Table 3.9.

Table 3.9. SED between histological foot scores from different factors

Experimental factors	SED
Mean scores on different litters	0.29
Mean scores from wet/dry treatments	0.42
Mean scores from day x litter	0.78
Mean scores from day x wet/dry treatments	0.59
Mean scores from litter x wet/dry treatments	0.83
Mean scores from day x litter x wet/dry treatments	1.10

At the start of the experimental period, birds reared on recycled cardboard chips, shredded recycled paper and woodchip all had similar mean histological scores (see Table 3.10.) of about 2.50. However, at the same time, birds reared on straw showed much more severe lesions, with a mean of 5.12. Birds reared on straw exhibited consistently higher histological foot scores throughout the experiment. Birds reared on recycled cardboard chips and recycled shredded paper had similar mean histological scores from about 2.50 and slowly increasing in severity with time. Woodchip as a litter resulted in birds having the mildest lesions. Birds had slightly higher histological foot scores at day 0, but scores did not increase as much as in birds housed on any of the 3 other litter types.

Birds reared on recycled cardboard showed similar histological foot scores on both wet and dry litter at days 0, 2 and 4. Histological scores had worsened on wet litter by day 6. Dry litter scores were still reasonably high at 5.75 on day 6.

Recycled paper as a litter produced similar histological scores at day 0 and 2. By day 4, histological foot scores were at least 2 points higher on wet litter than on dry. Paper bedding produced high histological scores by day 6 on wet litter, whilst dry litter resulted in low scores of 3.25.

Birds housed on straw as a bedding litter resulted in very high histological foot scores throughout the experiment. Scores at day 0 were at least 1 point higher than on other litters. By day 4, wet straw litter had a score of 6.50, higher than other all other litters by at least 0.75 points on the scoring system. These data suggest that straw is not a suitable litter for young turkeys as it resulted in high histological scores even when dry.

Birds housed on wet woodchip showed histological foot scores that worsened with time. There was a 3.25 point score increase from dry woodchip litter at day 0, to wet litter at day 6. It appears from these data that woodchip results in low foot scores if kept dry, but produces severe lesions, (mean score 6.25 by day 6) very rapidly if litter is wet.

At day 0 of the experimental period all histological foot scores were from birds housed on dry litter as any wet litter treatments had not yet been applied. All scores were low, i.e. below 4, which is a lesion of less than 1/8 total foot pad area. The only exception was straw, which showed a high result at day 0 with mean scores of over 4.5. By day 6, all wet litter showed high scores of over 6.25. It is concluded that it is wetness that is the cause of the lesion severity, rather than any difference in the type of litter used in this experiment, or any difference between litter type when wet or dry, or any irritant being released from woodchip when it becomes wet. The reason that straw caused such severe lesions may be due to the fact that it does not absorb moisture, and therefore the pen becomes water logged and dirty.

Table 3.10. Mean histological foot scores from birds housed on different litter types, both wet and dry, with time

Day Litter	Litter Condition	Day 0	Day 2	Day 4	Day 6
Recycled cardboard	Dry	2.50	5.00	5.25	5.75
	Wet	-	4.25	5.00	6.25
Recycled paper	Dry	2.12	4.25	3.50	3.25
	Wet	-	4.50	5.75	6.75
Straw	Dry	5.12	5.75	5.00	4.75
	Wet	-	5.00	6.50	6.25
Woodchip	Dry	2.75	3.75	3.25	3.00
	Wet	-	4.00	5.50	6.25

Litter ammonia levels

There was little difference in the logged ammonia readings (Table 3.11.) at days 0 (start of the experimental period, with birds aged 4 weeks) and 6 (end of the experimental period, birds, with aged 4 weeks and 6 days). All effects were small compared with their standard errors. Any small difference (*ca* 2-3 ppm) would be difficult to detect as the standard error (SED) was larger than the difference between treatments, and between wet and dry pens on the same day. The SED between mean scores is expressed in Table 3.12. There was a highly significant effect ($P < 0.001$) of day on litter ammonia scores, and an apparently significant effect of day x litter x wet/dry ($P = 0.05$). This significant difference between ammonia levels recorded from different litter types with time may have resulted from the low reading from wet cardboard at day 6. There were no other obvious significant factors.

Table 3.11. Mean logged and back transformed values for ammonia concentration (ppm) above litter within pens at day 0 & 6 of the experimental period (SED 0.48)

Litter type	Day 0 Dry (logged values)	Day 0 Dry (transformed values)	Day 6 Dry (logged values)	Day 6 Dry (transformed values)	Day 6 Wet (logged values)	Day 6 Wet (transformed values)
Recycled cardboard	0.48	1.62	0.97	2.63	1.01	2.74
Recycled paper	0.70	2.01	1.62	5.04	1.70	5.48
Straw	0.44	1.56	1.59	4.88	1.12	3.06
Woodchip	0.50	1.64	1.75	5.76	1.77	5.89

Table 3.12. SED between logged ammonia scores from different factors

Experimental factors	SED
Mean scores on different litters	0.32
Mean scores from wet/dry treatments	0.22
Mean scores from day x litter	0.34
Mean scores from day x wet/dry treatments	0.24
Mean scores from day x litter x wet/dry treatments	0.48

Discussion

Literature from previous experiments (see Chapter 1) suggested that wet litter had a significant effect on turkey foot pad dermatitis and resulted in higher external foot scores. (However, previous experiments did not distinguish between wet clean litter and wet dirty litter, that is, litter contaminated with faeces). The experiments reported in this chapter showed that wet litter alone had a significant effect on both external and histopathological foot scores, a fact that had not previously been reported. These experiments also indicated that ammonia levels emitted from different litter types were significantly different, however these significant differences may be due to the fact that the reading for cardboard at day 6 on both wet and dry litter was lower than on others litters. The fact that ammonia levels were lower from cardboard litter suggests that ammonia is unrelated to FPD, as similar foot scores were recorded from birds housed on all types of litter. Litter ammonia levels were similar on both wet and dry litter after 6 days. These data indicate that ammonia is unrelated to wetness. The findings of this work agreed with a previous report that wet litter showed no difference in litter ammonia levels (Wang *et al.* 1998), and contradicted claims made that wet litter showed higher ammonia levels compared with dry litter (Lumb 2002).

As time progressed, foot scores became more severe. The treatments caused more damage to the foot pads the longer the birds were housed upon them by increasing the severity of foot pad lesions.

There was a highly significant difference between foot scores (both histopathological and external) from birds housed on different litters, and as a result of housing on wet and dry litter. Foot scores from birds housed on dry litter were significantly lower than those reared on wet litter of the same litter type. This suggests that wetness is an important factor in the development and severity of FPD.

Birds housed in dry woodchip, recycled paper and recycled cardboard had similar histopathological and external foot scores throughout the experiment from day 0-6, and were significantly lower than for those reared on dry straw. These data suggest that straw is an inappropriate bedding material for young turkeys.

Although recycled cardboard appears to cause high histopathological and external foot scores in birds housed in dry litter at day 6, this may be due to the fact that recycled cardboard was extremely difficult to keep dry, resulting in slightly damp litter even when such pens were meant to be dry. At day 0, birds housed on dry cardboard had low foot scores similar to those found in birds housed on dry paper and woodchip bedding. This suggests that it was because the litter had become damp that caused a change in foot pad scores in these birds.

These data have shown that there is little difference in the logged ammonia readings from different litters within each sampling point, that is, at day 0, a similar level of ammonia was recorded from all 4 litter types. Similar results were gathered at day 6. Litter ammonia levels were higher at day 6 than at day 0, but there did not seem to be a difference in ammonia levels from wet and dry litters. However, it would be

difficult to detect any significant difference due to the fact that the standard error was larger than the difference between treatments, and between wet and dry pens on the same day. Ammonia has been suggested to be one of the factors involved in the development of FPD (Lumb 2002). Since ammonia scores were similar in all pens and within all treatments, this suggests that ammonia was not responsible for causing the range of foot scores recorded from birds housed on different treatments in these experiments.

The histopathological and external foot scores highlighted the fact that wetness is an important factor in the development of FPD. Birds housed on wet litter, regardless of litter type had significantly higher foot pad lesion scores. Birds reared on straw showed significantly higher lesion scores than those birds housed on other litters, confirming results from the external scores and the conclusion that straw is not a suitable litter for young turkeys. Birds reared on cardboard show high foot scores on wet and dry litter at day 6, although this may be due to the fact that the litter was not kept as dry as it should have been, as birds on dry cardboard at day 0 had low scores comparable to those scores found in birds housed on woodchip and paper.

Overall Discussion and Conclusion

From all three of these experiments investigating the effects of litter treatments on the development of turkey foot pad lesions it can be concluded that litter wetness is the most important factor in the development of lesions. Birds reared on wet litter developed lesions very rapidly within the experimental period. There does not appear to be a great deal of difference in the severity of lesions when birds were housed on different types of litter if wet and wetness had a greater effect than litter type. These results suggest that it is wetness rather than faeces in the litter, or any irritant released from the woodchip that were causing foot pad lesions. Straw as a litter resulted in more severe foot pad lesions than other litter, even when dry, suggesting that it is an unsuitable litter substrate for young turkeys (up to 5 weeks, as were used in these experiments). Since these experiments have suggested that it is wetness rather than litter type that causes the most severe lesions, a combination of litter types such as a lower layer of very absorbent material, topped with a layer of soft, non abrasive, but possibly less absorbent material may be a good solution to reducing friction between litter and skin (which may wear away when skin has been softened by wetness), whilst keeping absorbency high (Malone 1982).

Chapter 4

Effects of increasing dietary biotin concentration on turkey foot pad dermatitis

Introduction

Previous research reviewed in Chapter 1 has suggested that biotin plays an important role in skin formation, maintenance and repair (Harms & Simpson 1977; Buda 2000b). Some researchers have claimed that standard commercial diets for turkeys contain inadequate amounts of dietary biotin to prevent foot pad lesions (Richardson & Wilgus 1967; Misir & Blair 1988). Very high levels of dietary biotin (approximately 2000 μg/kg) reduced the severity of foot pad lesions in turkeys (Wakeman 2000; Buda 2000a; Buda 2000b).

The work reported here was designed to test the hypothesis that standard levels of inclusion of biotin (approximately 250-300 μg/kg) were inadequate in preventing foot pad lesions in commercial turkeys. These experiments also aimed to test claims that very high levels of biotin were able to prevent or reduce the severity of FPD.

Body weight, food intake, plasma biotin concentration, external and histopathological foot pad scores were obtained throughout the experiment at 2 week intervals. Liver samples were also taken at weeks 4, 8 and 14 and analysed for the fat percentage to observe any effects of biotin levels on fatty liver and kidney syndrome (FLKS), as low levels of biotin are known to be associated with FLKS and sudden death (Whitehead 1990). Litter moisture was determined for comparison between treatments and with other studies at 4, 8 and 14 weeks of age.

Materials and Methods

Birds

The experiment utilised 600 newly hatched male T8 Large White Broad Breasted turkeys supplied by Bernard Matthews', Norfolk, from an original breeding stock from B.U.T. The birds were fed a commercial pelleted ration specific to their age. There were 36 birds per pen at the start of the experiment.

Housing

Birds were housed from day old in 16 pens, each 3 m by 2 m. Each pen had the potential to double in size when the central divide was removed at 8 weeks of age allowing for the size of the pen to increase as the birds grew. The trial attempted to replicate commercial conditions, so the woodchip litter was kept as a relatively thin covering of approximately 2 cm over the concrete floor. The litter was kept damp, with new litter only added when the litter reached a score 4 or higher on Tucker & Walker's wetness score (see Chapter 3, Table 3.1.). Each pen contained a hanging bell drinker, a feed dish (for the first 28 days of the birds life), and a cylindrical food hopper, both containing the relevant diet. The pens were provided with a suspended heat lamp. From day 0, the light intensity was 10 lux, and the air temperature, 28°C. Treatments were allocated at random within 4 blocks. Each treatment was replicated 4 times.

Experimental treatment conditions

One of 4 different experimental diets was allocated to each pen (Table 4.1.). Diet recipes were devised by Mr Andrew Ball of Roche Vitamins Ltd (now DSM Nutritional Products, Derbyshire, UK) to reflect standard commercial diets used in the UK industry. Nutrient specifications were based on B.U.T. guidelines (British United Turkeys 2003). Diets contained varying amounts of biotin at 0, 200, 800 or 1600 μg/kg. Diet formulations changed after week 4 and week 8 to adjust to the birds growing needs, and the pellet size was increased. However, although diet formulations for crude protein were adjusted to the birds needs at week 4 and 8, the amount of biotin per kilogram remained the same within each diet.

Table 4.1. Feed ingredients and proximate compositions for the basal diets (Treatment 1) fed to turkeys at three different ages. Increasing quantities (0.5, 2.0 and 4.0 g/kg) of biotin in limestone flour replaced limestone to achieve 400, 800 and 1600 µg/kg supplemental biotin respectively in Treatments 2, 3 and 4.

Ingredient	Amounts of ingredients in each basal diet g/kg		
	Age diet fed		
	0-4 weeks[1]	5-8 weeks[2]	9-14 weeks[3]
Wheat	383	423	473
Fish meal	50	25	-
Field peas	25	40	60
Full fat soya	100	50	-
Hipro soya 48	368	351	331
Rape seed meal	14	40	40
Poultry fat blend	10	21	44
Vitamin-mineral premix[1]	12	10	8
Monocalcium phosphate	22	23	24
Limestone	7.5	7.8	10
Salt	-	1.3	2.4
Sodium bicarbonate	2.8	2.0	1.9
Lysine	2.0	2.0	1.8
Methionine	3.0	3.3	3.2
Threonine	0.7	0.6	1.0
Calculated composition			
Energy, MJ ME/kg	11.6	12.0	12.4
Crude protein, g/kg	294	264	230
Calcium, g/kg	14.0	13.0	12.0
Phosphorus, g/kg	10.0	10.0	9.0
Lysine, g/kg	1.82	1.61	1.36
Methionine, g/kg	0.74	0.71	0.63

Cycostat coccidiostat was added at 0.5 g/kg to each of the starter and grower diets.

[1] Supplied per kg of basal diet: Cu 15 mg, I 2.4 mg, Fe 50 mg, Mn 120 mg, Zn 84 mg, Se 0.3 mg; Mo 0.6 mg; Co 1.0 mg, retinyl acetate 4.6 mg, cholecalciferol 125 µg, α-tocopherol 100 mg, menadione 5 mg, riboflavin 15 mg, thiamine 5.0 mg, nicotinic acid 120 mg, calcium pantothenate 25 mg, pyridoxine 8 mg, cyanocobalamin 40 µg, folic acid 3.6 mg, choline chloride 400 mg.

[2] Supplied per kg of basal diet: Cu 12.5 mg, I 2.0 mg, Fe 42 mg, Mn 100 mg, Zn 70 mg, Se 0.25 mg; Mo 0.5 mg; Co 0.83 mg, retinyl acetate 3.9 mg, cholecalciferol 104 µg, α-tocopherol 83 mg, menadione 4.2 mg, riboflavin 12.5 mg, thiamine 4.20 mg, nicotinic acid 100 mg, calcium pantothenate 21 mg, pyridoxine 6.7 mg, cyanocobalamin 33 µg, folic acid 3.0 mg, choline chloride 333 mg.

[3] Supplied per kg of basal diet: Cu 10 mg, I 1.6 mg, Fe 33 mg, Mn 80 mg, Zn 56 mg, Se 0.20 mg; Mo 0.4 mg; Co 0.67 mg, retinyl acetate 3.1 mg, cholecalciferol 83 µg, α-tocopherol 67 mg, menadione 3.3 mg, riboflavin 10 mg, thiamine 3.3 mg, nicotinic acid 80 mg, calcium pantothenate 16.7 mg, pyridoxine 5.3 mg, cyanocobalamin 27 µg, folic acid 2.4 mg, choline chloride 267 mg.

Litter quality and sampling

Litter was monitored using the scale devised by Tucker & Walker (1999) outlined in Chapter 3 (Table 3.1.). Litter samples were taken at week 4, week 8 and week 14, when diets were changed. One small sample of litter was taken at 30 cm from each wall forming the corner of the pen. Samples were taken with a small garden trowel and mixed in a bucket. A small plastic tray was weighed, filled with the litter sample and reweighed. Samples were dried in an oven at 60°C for 2 weeks. Dried samples were weighed again and the percentage moisture loss calculated.

Culling of birds

Birds were restrained and injected intravenously into the brachial wing vein with approximately 2 ml of sodium pentaborbitone to ensure humane culling.

Collection of liver samples

Samples were taken from the same birds killed for histopathology at weeks 4, 8 and 14. After death, the tissue just below the breast bone of each bird was incised and the abdominal cavity opened. Small pieces of each bird's liver were removed with scissors, one piece of approximately 5 cm x 5 cm from each lobe of the liver. Both samples were stored in a small zip lock bag labelled with the bird number and frozen at –20°C.

Bird weight and food intake

Birds were individually weighed at 2 week intervals (weeks 2, 4, 6, 8, 10, 12, 14) and foot pads examined in order to assign an external foot pad score. One person (R.K.Mayne) carried out the foot pad scoring in order to reduce observer effects. The total weight of food consumed by each pen of birds was calculated by weighing food hoppers at the end of each week.

Collection of foot pad samples

Histopathological foot samples were taken from 2 randomly selected birds from each pen every 2 weeks, at the same time as birds were weighed.

Collection of blood samples

The same birds that were euthanased for histopathology samples, also had blood samples taken before euthanasia for blood biotin analysis. 2 ml of blood was extracted from each bird using a 0.5 mm needle, and stored in heparin treated tubes, then kept on a roller to prevent coagulation before freezing at –20°C.

Preparation of foot pad samples

After culling, the right footpad from each bird was scored externally as described in Chapter 2 (Table 2.1.), the skin of the footpad was then removed and stored in 10 % BNF. Sections were prepared and processed using standard protocols for section

processing (see Chapter 3). Sections were examined under the light microscope and categorised using the histopathological scale described in Chapter 2 (Table 2.2.).

Liver fat analysis

Individual frozen liver samples were removed from the bag and weighed. The bags containing the frozen liver samples were opened and placed into a freeze drying machine (Edwards Freeze Dryer, Super Modulyo. Edwards, Crawley, UK). The lid was fitted tightly and the vacuum pump turned on for 4 days to remove all moisture from the liver samples. When completely dried, the vacuum was broken by opening an air inlet, after which the samples were removed and stored at room temperature

Dried liver samples were manually crushed into a homogenous powder using a pestle and mortar, then approximately 1 g of powder was weighed into a small circular piece of filter paper. The sample weight was recorded and the remaining powdered liver sample was stored at room temperature. The filter paper was labelled with the bird number and folded into a small packet to ensure that no powder was able to escape. Folded samples were pushed into individual paper thimbles and topped with cotton wool to prevent any escaping powder, all within a metal rack in a fat extraction machine (Tecator Soxtec 1043 Extraction Unit. Tecator, Höganäs, Sweden). The rack was raised into the machine and held in place by a magnet. Metal cups in a rack below the paper thimbles were weighed individually and recorded against the bird number. Each cup was half filled with liquid petroleum, and the rack raised up so that each paper thimble was sitting within a metal cup half filled with petroleum spirit. The machine was then switched to the boiling position

for 1 hour, so that petroleum spirit was evaporated through the powdered liver, condensed and allowed to drip back through the samples. After 1 hour the machine was switched to the rinsing position and left for another hour. The paper thimbles were then raised up once more to enable the removal of the metal cups. The metal cups were placed into an oven overnight at 100°C to evaporate off the remaining petroleum spirit, leaving the fat in the bottom of the cups. The metal cups were placed into a dessicator and left to cool. Meanwhile, the paper thimbles were removed from the machine and left to dry on the bench top. The liver samples within the filter paper were then frozen at –20°C for later analysis if required.

When cooled, the metal cups were weighed and the weight of the cup plus the weight of the fat within the cup were recorded against the bird number from which the fat was extracted. The percentage fat from each sample could then be calculated. The metal cups were then filled with a strong detergent to remove the solid fat from the cups, left for 30 minutes to dissolve the fat, washed thoroughly and left to dry overnight in a drying cupboard before reuse.

The weight of the metal cup before the experimental procedure was subtracted from the weight of the metal cup plus the fat in the base after the fat extraction had taken place, giving a weight for the fat extracted from the liver sample. The weight of the fat was then divided by the dry weight of the sample at the start of the procedure, and multiplied by 100, to provide the percentage of the liver sample that had been extracted as fat.

Biotin plasma analyses

Samples were kept frozen until the end of the trial, when all samples were packed in dry ice and sent by courier to Roche Vitamins Ltd, Switzerland (now DSM Nutritional Products). Microbiological methods were employed to determine plasma biotin levels according to protocols published previously (Frigg & Brubacher 1976). Blood was centrifuged to extract plasma samples, then papain solution and volatile preservative were added before incubation overnight followed by autoclaving. The assay solution was then centrifuged and filtered. Assays were carried out using Bacto-Biotin Assay Medium, and the biotin content calculated using a calibration curve. Analyses provided a numerical value for the amount of biotin in each blood plasma sample (ng/L).

Statistics

External and histopathological foot scores were analysed using REML (residual maximum likelihood) within Genstat, since there was more than one source of variation in the dataset. A chi-squared distribution for Wald tests was generated as a result of Genstat REML analyses, and indicated the probability of significant effects due to treatment effects.

Bird weights were recorded in grams and transformed by using natural logarithms to normalise the residual errors (because the error variance was proportional to body weight). Analysis was then carried out using REML. Mean values were back transformed to provide estimates of bird weights in the scale of measurement.

Food consumption was calculated per bird per week from data gathered from pen food intake per week. Bird intake per week was then transformed to bird intake per fortnight within each pen and analysed using REML.

Food consumption per bird per week was calculated by the equation;

$$\frac{\text{Pen food intake/week}}{\text{Number of birds/pen within that week}} = \text{Bird food intake/week}$$

Bird intake per fortnight was calculated to provide bird intake across weeks 1 and 2, 3 and 4, 5 and 6 etc. Fortnightly bird intake per pen was calculated by the equation;

$$\frac{\text{Bird intake/pen/week(week1)+Bird intake/pen/week(week2)}}{2} = \text{Intake/bird/pen/2weeks}$$

The effects of diet and week on bird liver fat percentage were estimated using REML, taking account of pen, week within pen and bird within week and pen variation. Percentage fat was transformed to the logistic scale before analysis as residual plots indicated that this was necessary to give approximately constant variance. The logged values were then back transformed to provide an estimation of mean liver fat percentage.

Blood biotin levels were analysed using a split plot ANOVA, as these data had constant variance and a normal distribution as indicated by residual plots generated in Genstat.

Results

External foot scores

External foot score values were generated from all surviving birds, as all birds were examined every 2 weeks. Means are presented in Table 4.2. The chi-squared value generated by Genstat suggested that there was a highly significant ($P < 0.001$) effect of week on external foot scores with an SED of 0.13 and 6 degrees of freedom (df), but no effect from diet (SED = 0.20, df = 3), block (SED = 0.20, df = 3), or week x diet (SED = 0.31, df = 18).

Table 4.2. Mean external foot scores from birds provided with varying amounts of dietary biotin with time

Diet	Week						
	2	4	6	8	10	12	14
T1(0µg/kg biotin)	2.24	3.16	5.00	4.73	5.27	4.95	5.30
T2(200µg/kg biotin)	2.59	3.20	5.33	5.01	5.51	5.08	5.46
T3(800µg/kg biotin)	2.47	3.66	5.57	4.77	5.59	5.02	3.30
T4(1600µg/kg biotin)	2.45	3.34	5.42	4.55	4.93	4.36	5.43

Histopathological foot scores

Histopathological foot score values were generated from 32 birds at each 2 week sampling period (2 birds per pen). Means are presented below (Table 4.3.). The chi-squared value from the Genstat output indicated that there was a highly significant

($P < 0.001$) effect of week (SED = 0.38, df = 6) on histopathological foot scores, but no effect from diet (SED = 0.60, df = 3), block (SED = 0.60, df = 3), or week x block (SED = 0.89, df = 18).

Table 4.3. Mean histopathological foot scores from birds provided with varying amounts of dietary biotin with time

Diet	Week						
	2	4	6	8	10	12	14
T1(0µg/kg biotin)	2.63	3.94	4.00	4.88	5.46	6.13	6.25
T2(200µg/kg biotin)	3.00	3.13	3.46	5.50	5.86	6.54	7.00
T3(800µg/kg biotin)	2.25	5.13	4.27	4.50	5.92	6.38	5.88
T4(1600µg/kg biotin)	2.63	3.50	4.50	3.50	4.50	6.13	6.50

Bird weight

Mean logged weights are presented in Table 4.4. with back transformed means in Table 4.5. The chi-squared values suggested that there was a highly significant ($P < 0.001$) effect of week on logged bird weight (g) (SED = 0.008, df = 6), but no effect of diet (P=0.758) (SED = 0.02, df = 3), block (SED = 0.02, df =3), or week x diet (SED =0.02, df = 18).

Table 4.4. Mean logged weights (g) from birds provided with varying amounts of dietary biotin with time

Diet	Week						
	2	4	6	8	10	12	14
T1(0µg/kg biotin)	5.95	7.11	7.94	8.50	8.87	9.08	9.38
T2(200µg/kg biotin)	5.96	7.13	7.93	8.51	8.89	9.08	9.37
T3(800µg/kg biotin)	5.97	7.11	7.91	8.51	8.86	9.06	9.37
T4(1600µg/kg biotin)	5.95	7.11	7.91	8.48	8.86	9.06	9.38

Table 4.5. Mean back transformed weights (g) from birds provided with varying amounts of dietary biotin with time

Diet	Week						
	2	4	6	8	10	12	14
T1(0µg/kg biotin)	384	1227	2801	4894	7132	8745	11870
T2(200µg/kg biotin)	389	1250	2792	4955	7231	8737	11778
T3(800µg/kg biotin)	391	1218	2727	4971	7055	8575	11702
T4(1600µg/kg biotin)	385	1227	2718	4834	7018	8628	11803

Bird food consumption

Logged mean food intake is presented in Table 4.6. with back transformed values in Table 4.7. There was a highly significant effect of time on logged food intake per

bird per day ($P < 0.001$, SED = 0.03, df = 72) and no effect of diet ($P = 0.841$, SED = 0.02, df = 9) or diet x time ($P = 0.879$, SED = 0.02, df = 9) on the total amount of food the birds consumed.

Table 4.6. Logged values for bird intake (g/d) within each 2 week period and each diet

Diet	Week						
	2	4	6	8	10	12	14
T1(0µg/kg biotin)	-3.24	-2.35	-1.57	-1.17	-0.87	-0.55	-0.66
T2(200µg/kg biotin)	-3.26	-2.35	-1.66	-1.19	-0.89	-0.59	-0.64
T3(800µg/kg biotin)	-3.24	-2.32	-1.67	-1.16	-0.86	-0.57	-0.64
T4(1600µg/kg biotin)	-3.27	-2.36	-1.64	-1.10	-0.86	0.61	-0.61

Table 4.7. Back transformed means (from logged values) for bird intake (g/d) within each 2 week period and each diet

Diet	Week						
	2	4	6	8	10	12	14
T1(0µg/kg biotin)	0.040	0.100	0.208	0.310	0.419	0.579	0.518
T2(200µg/kg biotin)	0.039	0.100	0.190	0.305	0.413	0.556	0.529
T3(800µg/kg biotin)	0.040	0.100	0.188	0.312	0.425	0.564	0.528
T4(1600µg/kg biotin)	0.038	0.100	0.193	0.335	0.425	0.544	0.544

Liver fat percentage

There was a significant effect of week on the liver fat percentage from birds sampled ($P = 0.003$, SED = 0.1105,df = 2). There were no effects from diet ($P = 0.364$ SED = 0.1557, df = 3), or week x diet ($P = 0.200$, SED = 0.2351, df = 6) on liver fat percentages. Logged liver fat percentages are shown below (Table 4.8.), with back transformed means in Table 4.9.

Table 4.8. Logged values for liver fat percentage at weeks 4, 8 and 14 and within each diet

Diet	Week		
	4	8	14
T1(0µg/kg biotin)	-2.758	-3.017	-2.632
T2(200µg/kg biotin)	-2.525	-2.984	-2.925
T3(800µg/kg biotin)	-3.108	-3.099	-2.818
T4(1600µg/kg biotin)	-2.716	-3.365	-2.874

Table 4.9. Back transformed means (from logged values) for liver fat percentage at weeks 4, 8 and 14 and within each diet

Diet	Week		
	4	8	14
T1(0µg/kg biotin)	5.965	4.667	6.708
T2(200µg/kg biotin)	7.410	4.817	5.095
T3(800µg/kg biotin)	4.278	4.316	5.639
T4(1600µg/kg biotin)	6.206	3.342	5.345

Blood biotin analysis

Blood biotin levels are presented in Table 4.10. There was a highly significant effect of diet ($P < 0.001$, SED = 243.9, df = 9), week ($P < 0.001$, SED = 272.6, df = 56), and week x diet ($P < 0.001$, SED = 560.7, df = 64).

Table 4.10. Blood biotin (ng/l) means within each week and diet

Diet	Week						
	2	4	6	8	10	12	14
T1(0µg/kg biotin)	2143	2317	1648	1823	2540	1458	1553
T2(200µg/kg biotin)	2952	3158	2055	2703	2830	1880	1124
T3(800µg/kg biotin)	5816	3141	3280	3175	2772	1895	1972
T4(1600µg/kg biotin)	5969	7859	4198	4150	4285	3270	2910

Litter Moisture

There was a highly significant effect of week on litter moisture percentage ($P < 0.001$, SED = 1.31, df = 24), but no effects from diet ($P = 0.069$, SED = 1.81, df = 9) or week x diet ($P = 0.916$, SED = 2.81, df = 30). Mean litter moisture levels are shown below (Table 4.11.).

Table 4.11. Mean litter moisture (%) at weeks 4, 8, and 14

Diet	Litter moisture %		
	Week 4	Week 8	Week 14
T1(0µg/kg biotin)	35.1	44.5	51.0
T2(200µg/kg biotin)	39.0	48.6	56.4
T3(800µg/kg biotin)	39.5	45.8	52.5
T4(1600µg/kg biotin)	35.6	42.5	50.6

Discussion

External foot scores

The only factor that caused any significant difference ($P < 0.001$) in external foot scores was week (age). External foot scores increased gradually from a score of approximately 2.4 to 4.9 by week 14. Scores did not rise totally linearly: at particular time points, the mean external foot score within a pen appeared to drop, and then rise again by the next recording and it should be noted that all birds in the pen were scored so that sampling was not an issue in this experiment. The change in mean external foot scores occurred within all diets, and did not occur at the same weekly recording period with all diets. This may be due to the fact that some lesions healed slightly before becoming worse again with time with continued insult to the foot pads from wet and dirty litter.

From week 2 to week 4, external foot scores increased in severity by almost a whole foot score point, from approximately 2.4 to 3.3. From week 4 to 6, the mean score increased by two points from 3.3 to 5.3. After week 6, external scores continued to be recorded at similar levels. These results confirm findings from Chapter 2. Lesions showed the same time progression and similar FPD scores. Both data sets indicated that up until the age of 6 weeks foot pad lesions increased in severity very rapidly until a score 5 was reached (meaning that a quarter of the foot pad was necrotic). Once an external foot score of 5 was recorded, the development of the lesion appeared not to increase in severity, the area of necrosis simply became more extensive.

Histopathological foot scores

Histological foot scores were taken from 2 birds from each pen every 2 weeks, so these birds represented a sample of the population within each pen. Since each bird had to be killed for samples to be obtained, it was not possible to track the development of individual birds throughout the experiment. Therefore some individual variation may be expected.

The chi-squared value from the Genstat output indicated that there was a highly significant ($P < 0.001$) effect of week on histopathological foot scores as with the external foot scores, but no effect of diet ($P = 0.865$).

Histopathological foot scores increased rapidly, like external foot scores, from week 2, with a mean score across all diets of 2.6, to 3.9 by week 4. The mean histological foot scores after week 4, still rose steadily, but the increase in severity was less rapid. By week 6, mean histological foot score was 4.1 and increased slowly from week 4 until week 14, by which times mean scores were 6.4.

There was only one drop in foot scores (as occurred with the data from the external foot scores), which occurred at week 8 within birds on the T4 diet (1600 µg/kg biotin). The mean score decreased from 4.5 at week 6 to 3.5 at week 8, then rose to 4.5 again by week 10. This curious drop may be due to the fact that lesions did in fact start to heal in some birds. This might be related to the large amounts of biotin since biotin is well documented to be involved in skin formation and repair (Harms & Simpson 1977; Buda 2000b). It is interesting to note that foot scores only became

less severe in birds fed the high biotin diet, as this suggests that biotin may have a preventative effect on the development of lesions if the litter remained cleaner and dried. However, due to the fact that as the birds increased in size, the litter became dirtier and wetter. Continued insult to the footpads from damp and dirty litter may have prevented any further healing, resulting in foot pad lesion scores becoming worse once more. Further experimentation would be required to validate these suggestions. However it is possible that individual differences between randomly chosen birds may have resulted in chance sampling of particularly low foot pad scores.

Taken together, these data suggested that biotin has no effect on the development or severity of foot pads when young turkeys are housed on litter that is allowed to become damp and soiled.

Bird Weight

As with both types of foot score measurements, there was a highly significant ($P < 0.001$) effect of week but no effect of diet ($P = 0.758$) on body weight. Bird weight per bird per pen rose rapidly until week 6, after which time it increased steadily, but less rapidly than in the first few weeks. These data were within the guidelines published by B.U.T. (British United Turkeys 2003), and indicated that the turkeys were gaining weight as expected for their age and sex.

Bird food consumption

There was a significant effect of age on the amount of food the birds consumed. Bird food consumption at each recording period was within advisory guidelines from B.U.T. (British United Turkeys 2003), which suggested that these turkeys were consuming normal amounts of food for commercial birds.

Liver fat percentage

Liver fat percentage was an important measurement, as biotin deficiency is known to cause fatty liver and kidney syndrome (FLKS) in poultry (Whitehead 1990) and FLKS may result in sudden death. There was a significant effect of week on mean logged liver fat percentage. Means from week 8 appeared to be higher than both weeks 4 and 14. These differences may be due to individual bird differences.

There were no significant effects of diet on mean logged liver fat percentages. Since there were no significant differences in liver fat percentage due to diet, it can be concluded that high supplements of dietary biotin in commercial turkeys diets will not affect liver fat percentage. These results suggest that high dietary biotin supplements should not provide additional protection against the development of fatty liver and kidney syndrome (FLKS).

Blood biotin analysis

There was a significant effect of diet ($P < 0.001$) on blood biotin levels, as well as a significant effect of week ($P < 0.001$). Birds consuming diets containing higher

supplementations of biotin showed elevated levels of blood biotin, as would be expected, showing that the birds were obtaining the increased dietary biotin as part of their food.

There were some fluctuations within blood biotin levels, but mostly, the greater the dietary biotin consumed, the greater the blood biotin levels. There was a significant effect of week on blood biotin levels that differed with time. However, there was no pattern in the results and this may be due to chance since the sample size was small.

Litter Moisture

The data showed that litter wetness increased with time, from 4 to 14 weeks (Table 4.11.). The levels of moisture within each pen at each time period were similar. These results suggest that birds within all the pens were soiling the litter to the same extent. No water was added to these pens and the increasing litter moisture was simply the result of spilled water and faecal matter. This may be an important factor in devising a management plan for turkey litter within commercial situations. Since foot scores became more severe with time, it may be that increasing litter wetness may contribute to the development of these lesions. It is also important to note that the diets were formulated using soya which contains fats that are difficult to digest for the birds. Partially undigested fats are more likely to result in wet sticky droppings that may increase litter wetness.

Overall Discussion and Conclusions

From these data it can be concluded that litter wetness increased without any human intervention throughout the duration of this experiment. Possibly as a result of increasing litter wetness, external and histopathological foot scores became more severe. The results showed that dietary biotin had no effect on foot scores. Bird food consumption and bird weight were both within guidelines published by B.U.T. (British United Turkeys 2003), suggesting that dietary biotin had no effect on taste or satiety experienced by the birds. The overall pattern of results from liver fat analysis indicated that dietary biotin had little effect on the liver fat of the birds sampled. Blood biotin levels were observed to be higher in birds consuming greater amounts of dietary biotin. These data suggested that the birds were absorbing the biotin consumed, and were likely to be assimilating biotin for biological functions such as skin maintenance and repair. These data from this experiment do not support reports that high levels of dietary biotin (approximately 2000 μg/kg) reduced the severity of foot pad lesions in poultry (Wakeman 2000; Buda 2000a; Buda 2000b). One previous experiment by Jensen *et al.* (1970) supports this work, that increasing amounts of biotin supplements did not improve FPD scores. The condition of the litter was very damp and soiled, and even very high intakes of dietary biotin were unable to prevent FPD in these conditions. It may be that high levels of biotin may prevent or contribute to the healing of foot pad lesions if litter was kept dry and clean. Further investigation of this hypothesis would be necessary to validate such claims.

Chapter 5

Immune responses associated with turkey FPD

Introduction

Turkey FPD results in necrotic areas developing on the foot pad, accompanied by redness, swelling, and most likely, pain. Inflammation may be occurring in turkeys as a result of physical injury to the footpad or as a result of an allergic response to the litter substrate or some other environmental factor.

Cytokines are soluble chemical messengers that assist in the regulation of the innate and adaptive immune system. Cytokines bring about and are released when inflammation occurs, and are detectable in blood and tissue. Cytokines are produced and secreted by different cells in response to an antigen being presented to the immune system. After secretion, cytokines bind to specific cell surface receptors to activate other immune cells. Measurement of titres of specific cytokines indicates the level of inflammation and potential damage to the tissue.

The cellular composition of avian blood at various points of development and points of stress has been analysed. Heterophil (equivalent to mammalian neutrophils) to lymphocyte ratios decreased from hatch to 4 weeks in chickens (Zulkifli & Siegel 1994) and in mallard ducks (Fairbrother & O'Loughlin 1990). Heterophils respond to cytokine stimulation by carrying out different cell functions, as well as synthesising and releasing cytokines themselves. Heterophils also assist in host defences by phagocytosing foreign matter and mediating acute inflammation (Kogut *et al.* 2003).

There are relatively few specific monoclonal antibodies to chicken lymphocytes compared with mammals for laboratory-based investigation. However, B cells, CD4+ and CD8+ T lymphocytes, as well as macrophages have been identified using antibodies developed at the Institute for Animal Health (IAH), Berkshire, UK. CD4+ T lymphocytes are helper cells that through the production of specific cytokine subsets drive cell-mediated immune responses (Th1), or drive humoral immune responses (Th2), or act as regulatory cells (Th3). CD8+ T lymphocytes are cytotoxic T cells that kill cells infected with intracellular pathogens. B cells produce antibodies that are involved in humoral responses. Macrophages are phagocytic cells, derived from blood monocytes that process antigens and present them to T lymphocytes (Steven & Lowe 2000).

Macrophages are attracted to the site of injury, and secrete cytokines. Activated macrophages produce pro-inflammatory cytokines such as IL-1β, IL-6 and IL-8, promoting the inflammatory response (Steven & Lowe 2000). Other cytokines such as IL-10 and IL-13 promote antibody responses. IL-10 suppresses IFN-γ production by Th1 T cells, and down regulates pro-inflammatory cytokine production, whilst IFN-γ is the primary stimulator of macrophages and promotes cell mediated immunity (Abbas *et al.* 1997).

In this investigation cellular and cytokine responses were evaluated in foot pad tissue. qRT - PCR techniques were employed (as used by Kaiser *et al.* 2003) to measure mRNA levels of the cytokines IFN-γ, IL-1ß, IL-6, IL-8, IL-10 and IL-13,

and staining of turkey foot pad sections was carried out to identify CD4+ T lymphocytes, CD8+ T lymphocytes, macrophages and B cells.

An hypothesis was posed that a cell mediated response may have resulted from a water soluble irritant in the litter. Therefore, the aim of these experiments was two-fold, firstly to identify the cells and cytokines involved in turkey FPD. Secondly, these experiments aimed to record the recovery of FPD once the remaining birds were housed on clean dry litter.

Materials and Methods

The expression of pro-inflammatory cytokines (IL-1ß, IL-6, and IL-8), Th1 cytokines (IFN-γ), Th2 cytokines (IL-13) and a T regulatory cytokine (IL-10) were measured using quantitative RT-PCR techniques. Expression from ribosomal RNA (28S) was also measured for comparison and normalisation of the data. Methods were based on those used by Kaiser *et al.* (2003). Cross-reactivity of chicken and turkey IFN-γ was identified at IAH by Lawson *et al.* (2001), allowing for chicken primers and probes to be used in these experiments with turkeys. Fiona Powell (IAH) cloned other turkey cytokines (unpublished data) and demonstrated similar chicken-turkey cross-reactivity. When there was no cross-reactivity between chicken and turkey cytokines, Fiona Powell developed turkey specific primers. Sections were also stained for B lymphocytes, CD4+ T lymphoctyes, CD8+ T lymphocytes and macrophages, using chicken antibodies known to be cross-reactive with the turkey, also developed at IAH (Lawson *et al.* 2001).

Birds

72 newly-hatched female T8 Large White Broad Breasted turkeys from Bernard Matthews' Farms, Norfolk (breeding stock originally supplied by B.U.T.) were fed a standard commercial pellet ration suitable for birds aged 0-5 weeks of age.

Housing

Birds were housed on clean dry woodchip litter in a single large pen for the first week, then distributed on clean dry woodchip, 6 birds per pen across 12 pens, each 2 m by 3 m. Each pen contained a hanging bell drinker, a round feed dish placed on the pen floor contained the pelleted diet, and a suspended heat lamp for the first 7 days. The air temperature was 28°C throughout the experiment.

Lighting

Light conditions were maintained at a low intensity of 20 lux throughout the experiment to keep cannibalism or any pecking between birds to a minimum. The photoperiod schedule was 14 hours of light with 10 hours of darkness.

Experimental treatment conditions

There were two treatments, wet clean standard woodchip and dry clean standard woodchip, 6 pens of each, distributed randomly across the 12 pens. Woodchip was clean, having been vacuum packed in polythene wrap from the suppliers, but not sterilised. There were 2 blocks of 6 pens each, with each treatment being replicated 3 times within each block. After 48 h, 2 birds from each pen were killed for sampling, and the remaining birds on wet litter were transferred to the nearest dry pen, resulting in a total of 6 pens containing 8 birds each.

Litter quality

Litter was monitored using a scale devised by Tucker & Walker (1999) as detailed in Chapter 3 (Table 3.1.). Dirty litter containing excreta was replaced with clean throughout the experiment, even in wet pens, to maintain constant experimental conditions. Pens containing dry litter were maintained at a score 1 on this scale, by removing wet litter from these pens and replacing it with dry woodchip as required. Pens containing litter that was meant to be wet, were maintained at a score 4-5 by adding water daily. Water was distributed evenly across all litter within the pen using a 5 litre bucket. 2 buckets of water (approximately 10 l) were applied daily to maintain a litter score of 4.

Foot pad data recording

After 28 days of rearing on clean dry woodchip (day 0 of the experimental period), the birds were weighed, external foot pads examined and assigned a foot pad score using the external scoring system (described in Chapter 2, Table 2.1.) before the treatment litter was allocated to each pen. 48 h after treatments were applied, all birds were scored externally again. The birds in wet pens were then transferred onto clean dry litter (for 15 days). During this period, external foot pad scores were taken every 3 days.

Foot pad sample preparation

Two birds were randomly selected from each pen for each sampling point. Selected birds were culled using cervical dislocation at 0 h and 48 h after litter treatments were applied to pens. Birds were transferred to a dissecting table cleaned with RNAzap (Ambion, Cambridgeshire, UK) to remove all unwanted traces of RNA from other sources. Instruments and gloves were also treated with RNAzap, and between the dissection of each bird. The skin of the footpad was removed and cut into 2 pieces. One small piece of footpad, 5 mm x 5 mm, was placed on a round cork tile (20 mm x 3 mm), covered in OTC (BDH Laboratory Supplies, Dorset, UK) before snap freezing in liquid nitrogen and stored in a zip-lock bag labelled with bird number, date and researcher identification, then stored again in liquid nitrogen until it could be placed into a –80°C freezer. A second part of the foot pad was stored in RNAlater (Ambion, Cambridgeshire, UK) in a sterile 15 ml centrifuge tube and kept on ice until being transferred to a fridge at 4˚C.

Remaining birds were placed onto dry litter for 15 days. At the end of this period foot pad samples were taken from two randomly selected birds from each pen; one bird that had been exposed to 48 h on wet litter, and one bird that had remained on dry litter throughout the whole experimental period. Samples were stored in 10 % BNF until sections were cut, processed and stained with H & E stain.

Cutting of footpad sections for immunohistochemistry

Frozen tissue sections (N = 24) were removed from the –80ºC freezer and equilibrated with the chucks for 30 min in a Leica CM 1900 cryostat (Leica Microsystems, Wetzlar, Germany). After equilibration, each cork-mounted foot pad sample was secured onto a chuck with OCT and left to harden. The chuck-mounted sample was then secured longitudinally into the cryostat holder to ensure that the orientation of the section was the same for each sample for ease of comparison. 6 μm sections were cut from each foot pad block before collection onto a labelled slide. This procedure was replicated four times for each different stain. Slides were air dried at room temperature overnight then fixed in ice-cold ethanol for 10 min. Trays of slides were wrapped in plastic cling wrap, covered in aluminium foil and stored in the freezer at –80ºC until required for staining.

Immunohistochemistry staining procedure

Processed sections (6 μm) (N = 24) were circled with a hydrophobic pen and allowed to dry for 2 min. Foot pad sections were then rehydrated with 200 μl of PBS pipetted onto each sample and left for 5 min. Processed sections were stained using a Vectastain® ABC α-mouse IgG HPR staining kit (Vector Laboratories, California, USA), following the manufacturer's instructions. The monoclonal antibodies used were developed at IAH and are shown in Table 5.1.

Table 5.1. Antibodies used to stain different cell types in foot pad sections

Cell type to be stained	Antibody name (as used at IAH)	Dilution
CD4+	AV29	1:5
CD8+	11-39	1:5
Macrophage	KuL01	1:500
B cell	AV10	1:5

The NovaRED staining solution was made up from the kit (Vector NovaRED substrate kit (for peroxidase) SK-4800) (Vector Laboratories) to visualise different cell types. NovaRed (200 µl) was pipetted onto each sample and left for 30 s - 3 min depending on the colour intensity required. Slides were allowed to dry overnight before mounting with a cover slip using Surgipath Clearium Mounting Medium (Surgipath, Illinois, USA).

Preparation of foot pad mRNA for qRT-PCR

RNA extraction

Foot pad tissue (~30 mg) from each bird (N = 24) was homogenised in 600 µl lysis buffer (RLT, produced by Qiagen, Crawley, UK) using a bead mill (Retsch MM300. Retsch GmbH & Co, Hann, Germany). Complete disruption of tissue was ensured by using Qiagen's QIAshredder (Qiagen) following the manufacturer's instructions. Total RNA was then prepared from the homogenised tissues using an RNeasy mini kit (Qiagen) following the manufacturer's instructions. Purified RNA was eluted in 50 µl RNase-free water (supplied in the kit) and stored at -70°C in a 2 ml tube labelled with the bird identification number.

Real-time quantitative RT-PCR

Real-time quantitative RT-PCR (using Taqman technology, Taqman (ABI Prism™ 7700 Sequence Detector, Applied Biosystems, California, USA)) was performed using the Reverse Transcriptase qPCR Master Mix RT-PCR kit (Eurogentec, Seraing, Belgium). Quantification was based on the increased fluorescence detected due to hydrolysis of the target-specific probes by the 5'-exonuclease activity of the rTth DNA polymerase during PCR amplification. The passive reference dye 6-carboxy-χ-rhodamine, which is not involved in amplification, was used for normalization of the reporter signal. Volumes of different components required for Taqman analysis are stated in Table 5.2. Primers and probes for use with the chicken (that were cross reactive with the turkey) were developed by Dr. Pete Kaiser and colleagues at IAH. Turkey-specific primers and probes were developed by Fiona Powell at IAH. Primers and optimal concentrations for Taqman analysis are detailed in Table 5.3. Turkey foot pad samples were analysed to measure mRNA levels of IL-1β, IL-6, IL-8 (pro-inflammatory cytokines), plus IFN-γ (Th1), IL-13 (Th2), and IL-10 (Treg).

Table 5.2. Volumes of components required for Taqman analysis

Reagents required for Taqman analysis	Per reaction (µl)
2x PCR Master mix (in Eurogentec kit)	12.5
Primer mix (at optimal conc^{n}.)	1.00
Probe	0.50
"Euroscript" Enzyme	0.125
RNase-free H_2O	5.875

Table 5.3. Primers and optimal concentrations for Taqman analysis

Cytokine	Turkey-specific or cross reactive chicken primer	Primer concentration (uM)
28S	chicken	0.6
IFN-γ	turkey	0.6
IL-1ß	chicken	0.4
IL-6	chicken	0.2
IL-8	turkey	0.6
IL-10	chicken	0.4
IL-13	turkey	0.4

5.0 µl RNase-free H_2O was added to each of the non-template controls (NTC) wells, followed by 20 µl Master mix. The NTC wells were then capped off to prevent contamination of the NTC.

For test samples and positive "standard" RNA, the RNA was diluted with Rnase-free water, 1:1000 for ribosomal (28S) analysis, or 1:10 for cytokine expression analysis. 15 µl diluted RNA was required for each sample (5.0 µl/well, triplicate wells).

For standard RNA for 28s and IL-1β, the dilution series were $1:10^3$, $1:10^4$, $1:10^5$, $1:10^6$ and $1:10^7$. For standard RNA dilutions for all other cytokines tested (IFN-γ, IL-6, IL-8, IL-10 and IL-13) the serial dilutions were $1:10^2$, $1:10^3$, $1:10^4$, $1:10^5$ and $1:10^6$. 20 µl of Master mix was added to all wells being used in the plate, and then 5.0 µl sample RNA was pipetted into well. All wells were capped off properly and the plate was spun down before analysis. The following cycle profile was used; one cycle of 50°C for 2 min, 60°C for 30 min, and 95°C for 5 min, then 40 cycles of 94°C for 20 s, followed by 59°C for 1 min.

Statistical analysis

Foot pad scores recorded from birds placed on dry litter for 15 days (after an initial 48 hr on wet litter) were scored using the external foot pad scale. Slides stained for specific cell types, and foot pad sections stained with H & E were examined under a light microscope and all positive staining was recorded. Results were analysed using a general split plot ANOVA in the statistical programme Genstat.

Analysis of data produced from Taqman runs

Quantification was based on the increased fluorescence detected due to hydrolysis of the target-specific probes by the 5'-exonuclease activity of the rTth DNA polymerase during PCR amplification. The passive reference dye 6-carboxy-χ-rhodamine, which is not involved in amplification, was used for normalization of the reporter signal. Results are expressed in terms of the threshold cycle value (C_t), the cycle at which the change in the reporter dye passes a significance threshold (ΔR_n).

To account for variation in sampling and RNA preparation, the C_t values for cytokine-specific product for each sample were standardised using the C_t value of 28S rRNA product for the same sample from the reaction run simultaneously. To normalise RNA levels between samples within an experiment, the mean C_t value for 28S rRNA-specific product was calculated by pooling values from all samples in that experiment. Tube to tube variations in 28S rRNA C_t values about the experimental mean were calculated. The slope of the 28S rRNA $\log_{10}$ dilution series regression

line was used to calculate differences in input total RNA. Using the slopes of the respective cytokine $\log_{10}$ dilution series regression lines, the difference in input total RNA, as represented by the 28S rRNA, was then used to adjust cytokine-specific C_t values, as follows:

Corrected Ct value = Ct + (Nt-Ct')* S/S'

Where Ct = mean sample Ct
Nt = experimental 28S mean
Ct'= mean 28S of sample
S = cytokine slope
S'= 28S slope

Results were then expressed as 40-C_t values and analysed using a general split plot ANOVA in the statistical programme Genstat.

Results

Cell staining data from birds sampled at 48 h

12 turkey foot pads were used for immunohistochemistry staining at 0h, and a further 12 birds at 48 h. No staining for B cells in any of the sections was observed. Positive staining for macrophages was seen in 10 of the 12 footpads from birds housed on wet litter within the uppermost layer of the epidermis just below the keratin layer (surface keratin had been lost), whilst there was no staining for macrophages in birds housed on dry litter. CD4+ T lymphocytes were evident throughout the upper and lower dermis of all birds housed on wet litter with scant evidence of positive staining in birds housed on dry litter (1 bird stained positive for CD4+ T lymphocytes). In birds housed on wet litter, 4 stained positive for CD8+ T lymphocytes.

Images of immunohistochemically stained sections

Sections were taken from birds housed on dry and wet litter for 48 h and stained for 4 different cell types. Sections were stained for B cells (Fig 5.1a. and 5.1b.), macrophages (Fig 5.2a., 5.2b and 5.2c), CD4+ T lymphocytes (Fig 5.3a., Fig 5.3b. and 5.3c), and CD8+ T lymphocytes (Fig 5.4a., 5.4b. and 5.4c). Any positive staining resulted in cells being stained very dark red-brown. Artefacts of processing which were likely to be contaminants, were present in most sections from birds housed both on wet and dry litter, appearing as large black particles, greater in size and darker in colour than positively stained cells. Artefacts of processing also had an uneven edge, whilst cells were more regularly shaped. The pale brown background stain on all sections was non-specific.

KEY

A: Keratin

B: Surface keratin has been lost

C: Epidermis

D: Dermis

E: Positively stained cells in epidermis and dermis

Stained for B cells in turkey foot pads (No positive staining)

Figure 5.1a. Turkey housed on dry litter (magnification x10)

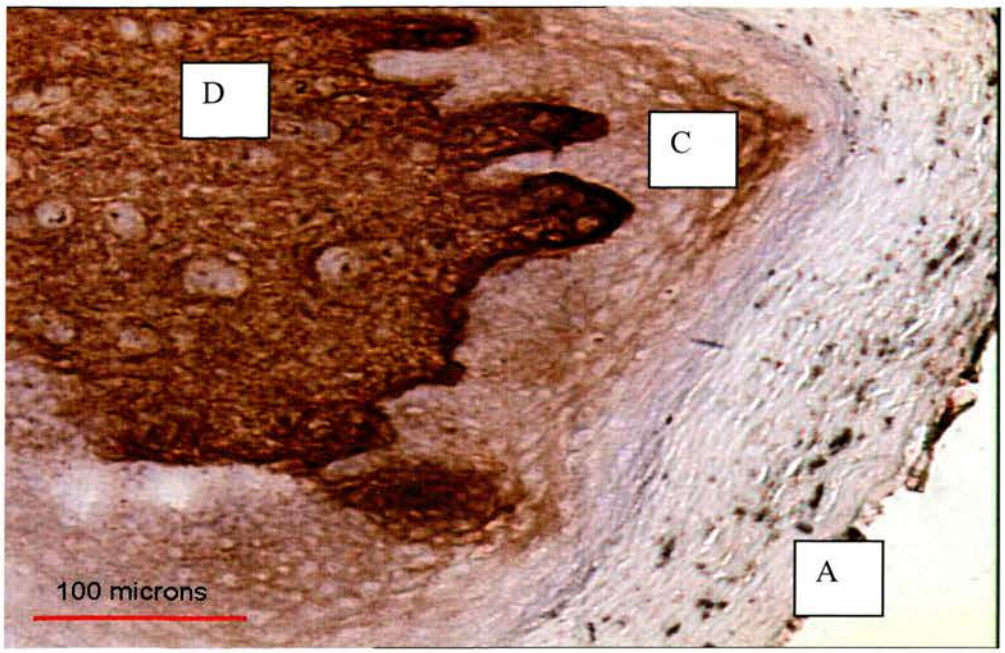
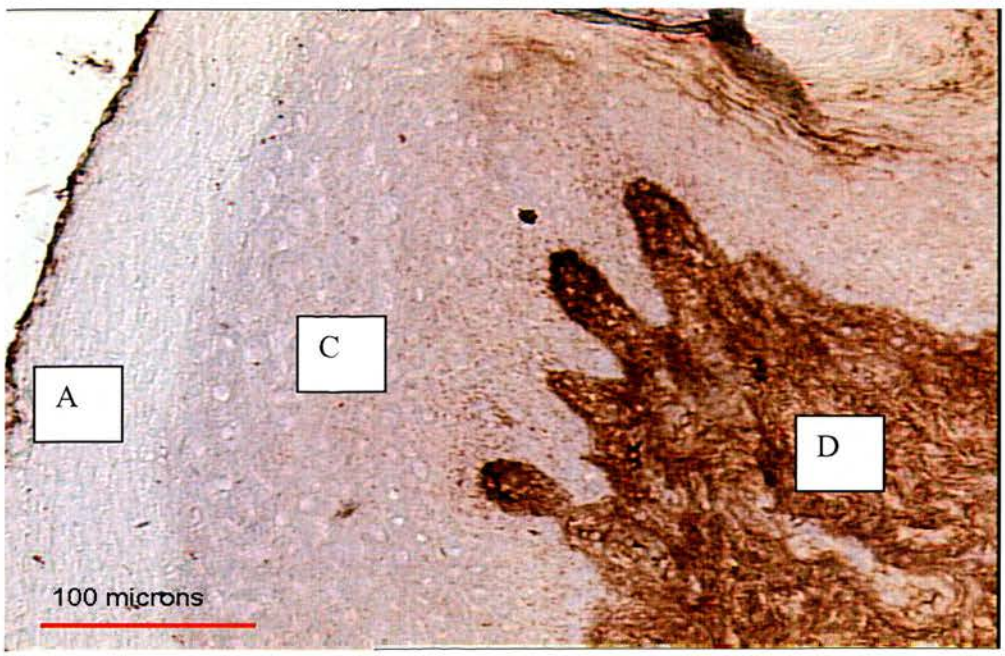


Figure 5.1b. Turkey housed on wet litter (magnification x10)



Stained for macrophages (positive staining in birds housed on wet litter)

Figure 5.2a. Turkey housed on dry litter stained for macrophages (magnification x 10)

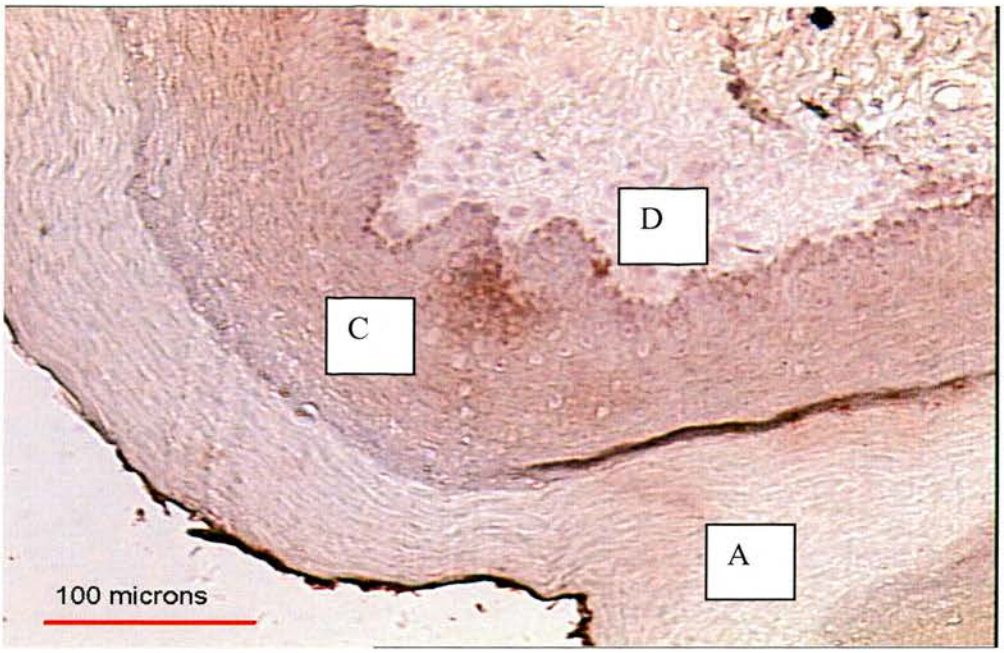


Figure 5.2b. Turkey housed on wet litter stained for macrophages (magnification x 10)

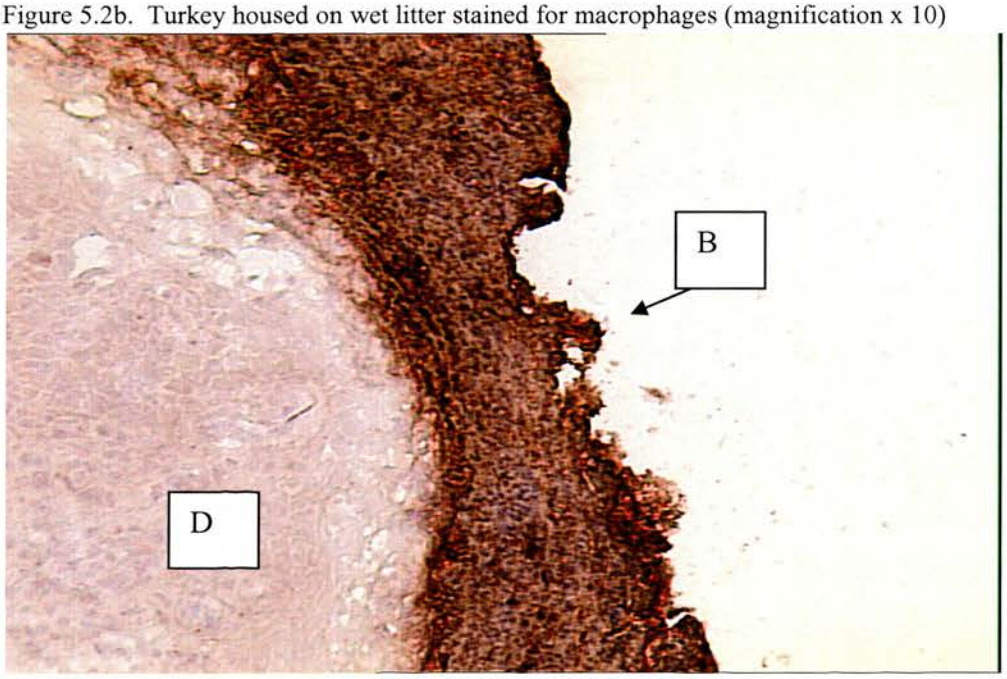
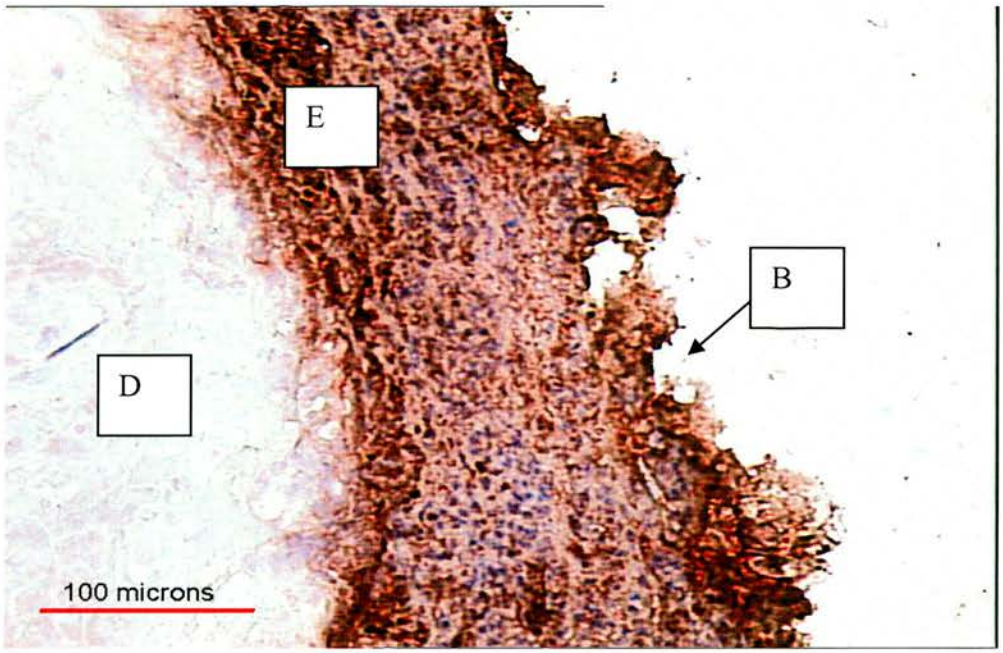


Figure 5.2c. Turkey housed on wet litter stained for macrophages (magnification x 20)



Stained for CD4+ T lymphocytes (positive staining in birds housed on wet litter)

Figure 5.3a. Turkey housed on dry litter stained for CD4+ T lymphocytes (magnification x 10)

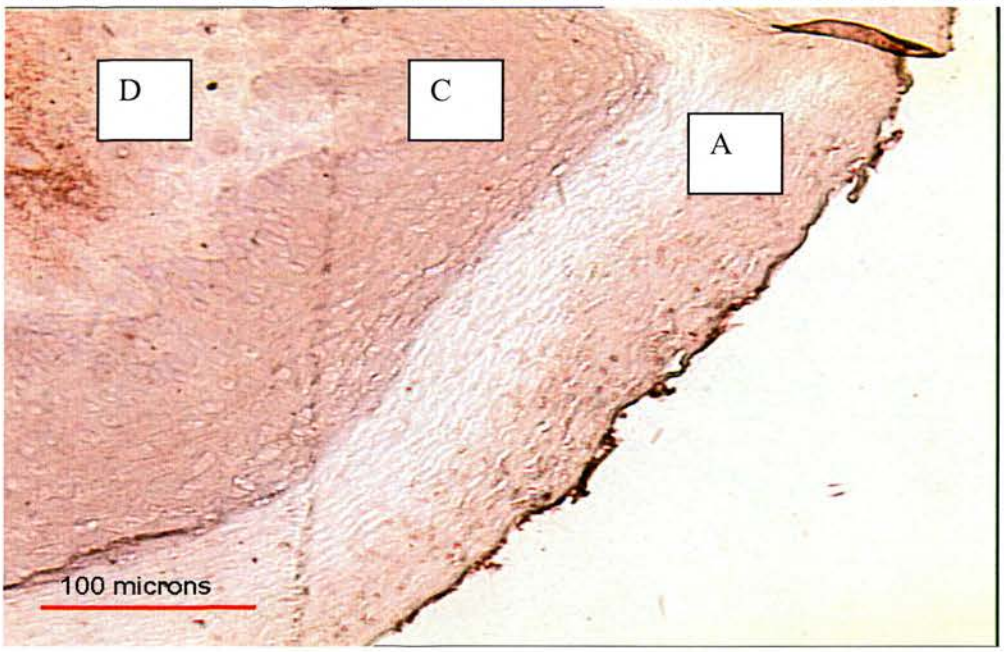


Figure 5.3b. Turkey housed on wet litter stained for CD4+ T lymphocytes (magnification x 10)

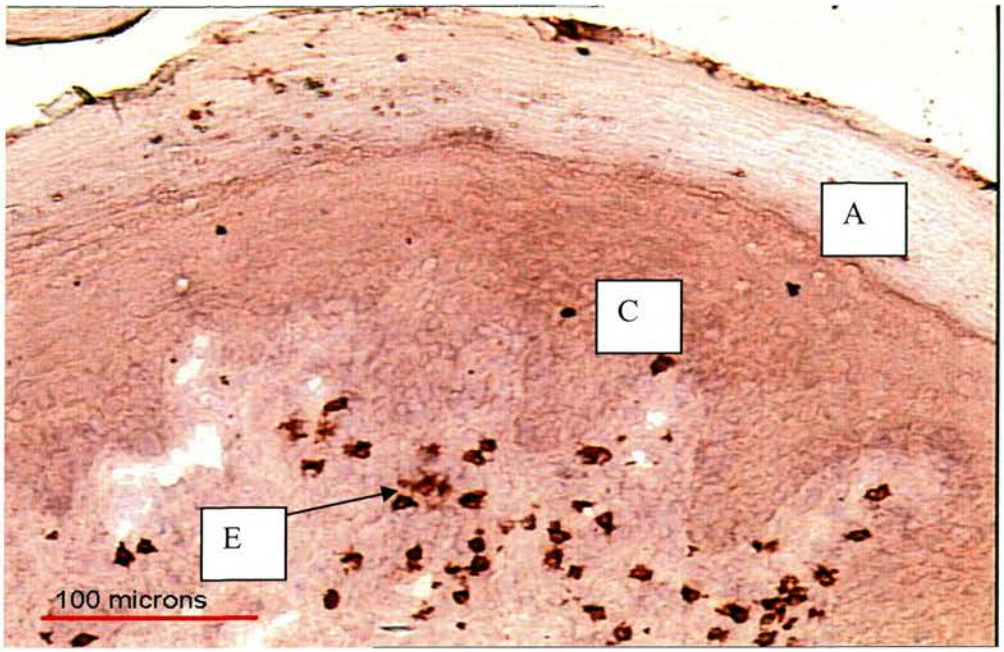
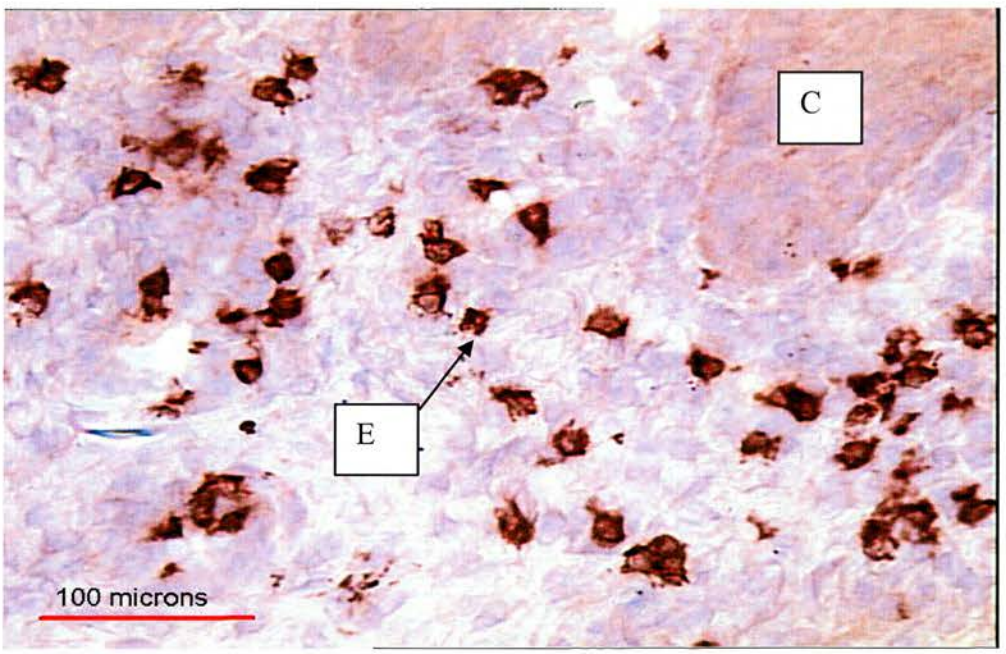


Figure 5.3c. Turkey housed on wet litter stained for CD4+ T lymphocytes (magnification x 20)



Stained for CD8+ T lymphocytes (positive staining in birds housed on wet litter)

Figure 5.4a. Turkey housed on dry litter stained for CD8+ T lymphocytes (magnification x10)

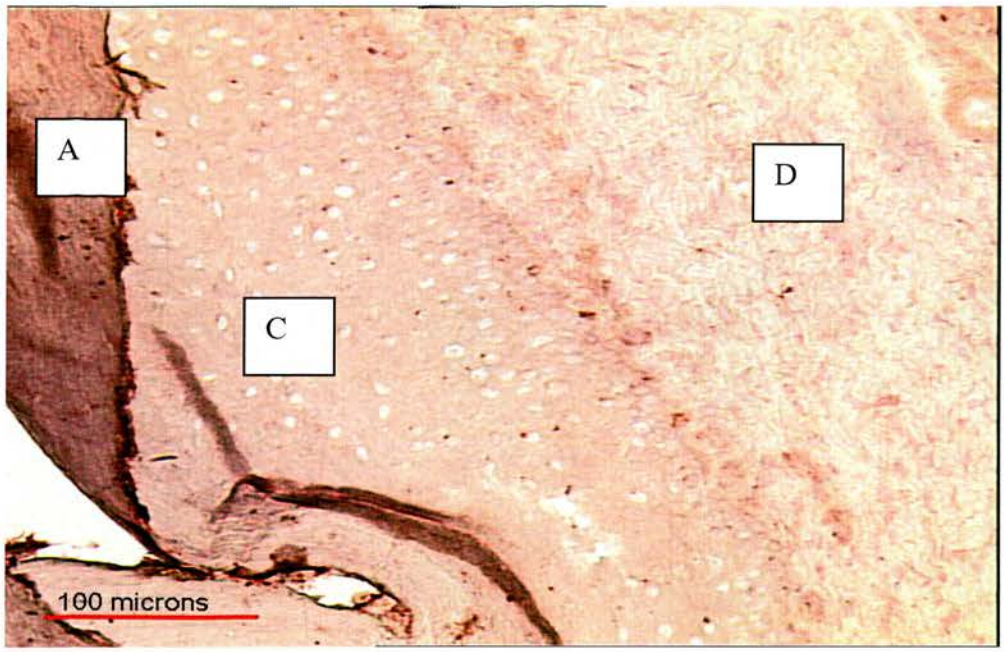


Figure 5.4b. Turkey housed on wet litter stained for CD8+ T lymphocytes (magnification x10)

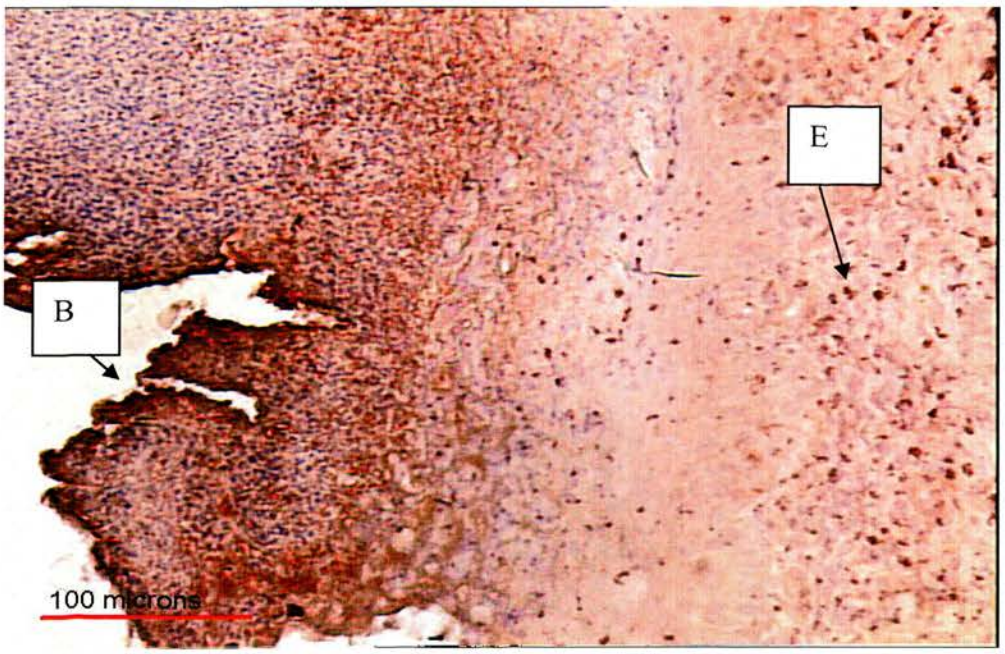
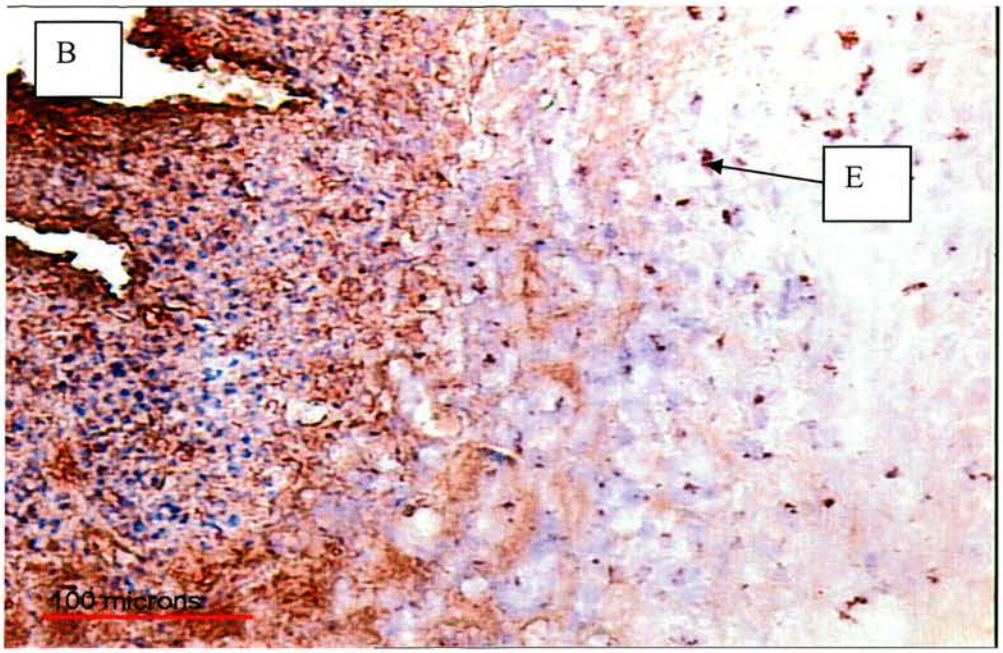


Figure 5.4c. Turkey housed on wet litter stained for CD8+ T lymphocytes (magnification x 20)



Foot pad data from birds sampled 15 days post-dry litter

Taqman data

Corrected C_t values from each cytokine measured (in triplicate for each bird) were analysed, using a split plot ANOVA within Genstat (Table 5.4.).

Table 5.4. Mean corrected cytokine C_t values (40- C_t) after analysis using ANOVA (N = 24)

Cytokine	Corrected C_t value from birds housed on dry litter	Corrected C_t value from birds housed on wet litter	S.E.D value	P value
IFN-γ	1.44	6.45	0.563	<0.001
IL1ß	3.66	12.97	0.854	<0.001
IL6	7.50	10.51	1.057	0.019
IL8	10.10	21.01	1.014	<0.001
IL10	4.02	2.51	0.780	0.086
IL13	7.43	8.52	0.262	0.002

There was a significant difference ($P < 0.019$) between the expression of the majority of cytokines measured (IFN-γ, IL1ß, IL6 and IL8) from birds housed on wet and dry litter after 48 hours. The differences in cytokine expression between birds housed on wet and dry litter were relatively small for IL-10 and IL-13 (the maximum difference was 1.51 +/- 0.780, and the probability value for IL-10 was 0.086). The differences between C_t values from IFN-γ, IL-1ß, IL-6 and IL-8 were considerably greater, from 5.01+/- 0.563 to a maximum 10.91+/- 1.014. The probability values from these analyses were highly significant ($P < 0.001$) (see Table 5.4.).

External foot pad scores

After 48 h of litter treatment, all birds were placed onto dry litter. External foot pad scores were taken at days 0, 3, 6, 9, 12, 15, and analysed using ANOVA (Table 5.5.). Scores at day 0 were 0.83 for birds housed on dry litter, and 6.89 for those housed on wet litter. Birds scored at day 0 were greater in number (72) than those scored on subsequent days (36), so day 0 scores were not included in the analysis. There was a highly significant difference ($P < 0.001$) between mean external foot scores recorded on different days and between treatments.

Table 5.5. Mean external foot pad scores recorded after placing on dry litter (SED 0.44)

Day	Original Treatment	
	Dry	Wet
3	2.44	6.78
6	0.22	6.33
9	0.28	5.17
12	0.39	3.06
15	0.00	1.39

The high score observed at day 3 from birds on dry litter may have been a result of the fact that a recording of a score 3 requires a focal patch of necrosis on the foot pad; a small piece of compacted woodchip and faeces that could not be removed could also be confused with a necrotic patch of skin. Since external foot scores on dry litter began at 0.83 at day 0, and were recorded as 0.22 at day 6, it seems likely that the higher foot score at day 3 was due to a categorisation error rather than an increase in FPD scores.

By day 15, mean external foot scores recorded from birds originally housed on wet litter had fallen from 6.89 to 1.39, indicating that healing of skin had occurred. However, the mean value was still significantly greater than those scores from birds housed on dry litter throughout the experiment (0.00).

Histological foot pad scores

Birds sampled 15 days after being placed on dry litter showed evidence of lesions healing. There was a significant difference (P = 0.01) between histological foot scores recorded from birds housed originally on wet or dry litter for 48 h, followed by 15 days housing on dry litter (Table 5.6.).

Table 5.6. Mean histological foot pad scores recorded after placing on dry litter (SED 0.67)

Day	Original Treatment	
	Dry	Wet
15	1.17	3.67

Histological lesions showed that birds housed on dry litter throughout the experiment were either normal or with minor cellular changes. Birds housed on wet litter for 48 h followed by 15 days on dry litter showed cellular changes such as the influx of inflammatory cells (heterophils and macrophages), increased blood vessel density, apoptotic nuclei within the keratin and epidermis, and vacuoles (hydropic change) within the epidermis. These changes had previously only been observed during the development of a lesion, however, in this case, it is more likely that the foot pads were healing, and returning to a normal state, since these birds had previously shown widespread necrosis of foot pad tissue, indicating severe lesions. The fact that these

sections showed only minor cellular changes and no evidence of any rupturing of the epidermis, as is seen in severe foot pad lesions, suggests that healing had occurred very rapidly, within the 15 days that the birds had been housed on dry litter.

Discussion

Evidence from sections stained for specific cell types showed that birds housed on wet litter had increased levels of macrophages, CD4+ and CD8+ T lymphocytes. In areas of high concentration of positively stained cells, higher magnification confirmed that the darker staining was in fact positively stained cells (Figures 5.2c., 5.3c. and 5.4c.). These results were consistent with the conclusion that there was a simple non-specific inflammatory reaction occurring within the foot pad of turkeys housed on wet litter for 48 h. It is unlikely that an allergic reaction could occur in such a short space of time (48 h) unless the turkeys had been previously exposed to the allergen causing the reaction. Also the cytokines that may indicate allergy were not recorded in the high levels which would be expected in the case of an allergic response.

Results showed that there was a significant difference in mRNA cytokine expression between birds housed on wet and dry litter after 48 h. The differences in mRNA expression of IL-10 and IL-13 from birds on wet and dry litter were very small, and in the case of IL-10, not significant at the 5 % level. IL-13 showed a statistically significant but not necessarily biological significant difference, as the difference was small. The differences in expression in IL-1β, IL-6, IL-8 and IFN-γ were greater, suggesting that there may be a real difference in expression in birds housed on wet and dry litter. The higher expression of IL-1β, IL-6 and IL-8 in birds housed on wet litter was also consistent with an inflammatory response. Positive staining for macrophages in sections taken from birds housed on wet litter supported this conclusion. Immunohistochemistry results indicated that there was positive staining

for CD4+ T lymphocytes in tissue from all birds housed on wet litter for 48 h, indicating a definite link between housing birds on wet litter and the production of CD4+ T lymphocytes within turkey foot pad tissue. Only 4 of the 12 birds housed on wet litter showed positive staining for CD8+ T lymphocytes. Since the observation of these cells was only present in 4 birds, this may be due to chance. However, since other inflammatory cells (macrophages and CD4+ T lymphocytes) had been recorded in tissue from birds housed on wet litter, it is possible that although CD8+ T lymphocytes were present in only 4 birds housed on wet litter, wet litter may be causing a proliferation of these cells in some birds before others. Naïve T lymphocytes usually take 4-5 days to respond so although these cells were present in the foot pad sections, they may not yet have been producing IFN-γ. A more likely scenario would be that the measured IFN-γ mRNA was produced by NK cells as part of the innate immune response (Personal Communication, Lisa Rothwell, IAH, 2005).

Positive staining for T lymphocytes (CD4+ and some CD8+) in stained foot pad sections, indicated that there may have been a cell mediated immune response taking place in the foot pad. Since T lymphocytes were evident in foot pad sections, it is possible that there was an antigen present in the birds' environment causing a proliferation of these cell types. Or there may have been a genetic or dietary reason for an increase in these cell types. The fact that wet litter caused higher numbers of T lymphocytes to be stained within the foot pad, whilst dry litter did not, suggested that there may be a water-soluble antigen being released from the woodchip used as litter. However, since such a reaction is unlikely to occur in such a short time frame

(Personal Communication, Lisa Rothwell, 2005), these results may be due to another unrelated factor. These results show a definite indication of non-specific inflammation, but as yet, no specific cell-mediated immune response can be confirmed.

Histology sections indicated that 48 h of being housed on wet litter caused birds to develop severe foot pad lesions. After housing thereafter on dry litter for 15 days, these lesions had almost totally healed externally, with only minor microscopical cellular changes, similar to those seen when lesions were starting to develop. Birds that had been housed on dry litter throughout the experiment had a small degree of cellular abnormality (such as hyperkeratosis, epithelial hyperplasia, epidermal acanthosis and increased dermal blood vessel density. (Histopathological scale, Chapter 2, Table 2.2.)). These changes were less extensive than those observed in birds that had been housed on wet litter. These data indicated that wet litter was the most likely cause of the inflammation within the foot pads of turkeys. Inflammation occurred extremely rapidly, within 48 h, and healed quickly also. Although the difference between mean histological foot scores was significant after 15 days of housing on wet litter, the difference was small. Externally lesions had formed into scabs, so scoring was based on area of the scab. Once scabs fell off, a small depression remained underneath. If birds were allowed to remain on dry litter for a longer period, lesions would probably have healed completely. Whether it was the wetness directly, or a soluble factor being released from the wet litter is as yet unclear, but it is highly likely that housing turkeys on wet litter was directly related to the development of severe foot pad lesions.

Birds on wet litter showed a disinclination to walk, spending as long as possible either standing in one place or sitting. These birds demonstrated bowed heads, raised feathers and closed eyes, compared with birds on dry litter that showed none of these behavioural traits. When walking, birds on wet litter were reluctant to place the foot on the ground. Birds lifted one foot, then spent longer with the foot raised off the ground than non-lesioned birds. When placing the foot on the ground again, the bird appeared very tentative, delicately finding a flat, soft piece of ground before placing the full weight on the foot. Birds housed on dry litter did not show such behaviour, simply placing one foot on the ground after the other without hesitation. After 24 hours on dry litter, the severe redness of the birds' feet that had been on wet litter showed reduced intensity. Necrosis of the epidermis and superficial dermis was still evident, but the redness was more of a subtle pink surrounding the necrotic areas. Birds seemed more content, feathers were cleaner, and there were no behavioural differences between birds that had previously been housed on wet and dry litter. These observations indicate that a welfare issue is involved with housing birds on wet litter, and that since birds were reluctant to walk there may be pain associated with FPD.

In conclusion, these data suggest that there was a rapidly occurring inflammatory response in the foot pads of birds housed on wet litter for 48 h. This inflammatory response caused cellular changes, characterised by an increase in blood vessel density, an influx of inflammatory cells into the dermis and a ruptured epidermis. These changes had reduced considerably within 15 days of housing on dry litter.

This experimental study examined birds housed on wet litter for 48 h. An extended study that measured cytokine expression and cell types at different time points would provide a more detailed picture of the cellular events taking place within affected foot pads. Staining for different cell types and testing for expression of different cytokines would also clarify these initial findings. It would be interesting to induce lesions, then house the birds on clean dry litter for longer than the 15 days in this experiment to observe if lesions healed fully on a histological level, returning to a completely normal state. Another important welfare experiment would examine pain in birds with FPD by recording behavioural patterns when birds with FPD are injected with local anaesthesia. These experiments would allow for an objective assessment of pain and associated behavioural responses.

Chapter 6

General Discussion

The experimental work carried out as part of this PhD has covered several aspects of the development of turkey FPD. The results from this work led to the conclusion that wetness of the litter can induce severe FPD lesions in young turkeys within a very short space of time (less than 48 h) in the absence of significant excreta. The practical outcome of this research is that litter must be better managed to improve turkey welfare and decrease the prevalence of FPD.

Previous to this work, no model had been reported that enabled FPD to be induced. These experiments provided a method by which FPD could be induced rapidly, which allowed meaningful observations and measurements to be made. When birds were housed on wet litter, inflammatory responses became apparent after just 24 h and the severity of the lesions increased with time. The use of this model has markedly improved the understanding of the development of FPD.

Although biotin showed no obvious effect on FPD, diet may play an important role in the development of this condition since litter wetness has been shown to be affected by dietary components, such as high levels of protein, and indigestible fats (Leeson & Summers 1991; Boling & Firman 1997; Tucker & Walker 1999; Murakami *et al.* 2000; Berg & Algers 2004).

Chapter 1 of this thesis examined experimental work that had already been reported in the scientific literature, which formed the basis of the subsequent experimental work carried out in this thesis. Field work carried out at one of the major commercial turkey producers within the UK provided information about how rapidly

turkey FPD occurs, and the cellular changes that take place. The survey highlighted the fact that birds as young as 3 weeks exhibited fully developed lesions. This field work also allowed for the development of scoring systems, both for scoring of external lesions on footpads as well as histological sections of foot pads microscopically. These scoring systems were then used throughout the experimental work that followed, allowing for comparisons between birds in individual experiments and between different experiments to be made easily.

Birds were placed on wet litter to induce lesions and the induced lesions healed rapidly when birds were placed onto dry litter. In foot pad sections taken after 15 days, the skin of the central area of the foot pad had returned to a pre-lesional state, although the difference between birds housed on wet and dry litter was still statistically significant and cellular changes were slight. These data help to confirm that wet litter is the primary cause of FPD, and that good litter management can reverse the damage caused by such environmental conditions even once FPD has resulted in severe lesions.

Cell types involved in the development of FPD were identified under the microscope using H & E stain, most notably the presence of heterophils and eosinophils. Heterophils are known to respond to cytokine stimulation in avians (Kogut *et al.* 2003). Other cells identified were macrophages and T cells (CD4+ and CD8+) which were identified using immunohistochemical staining. The presence of these cells in birds affected by FPD suggested that there was a specific cell-mediated inflammatory response occurring within the skin of the foot pad.

qRT-PCR techniques were employed to identify cytokine expression within turkey foot pad skin in Chapter 5. The most notable differences were between the expression of IFN-γ, IL-1ß, IL-6 and IL-8. The expression of these cytokines was considerably greater in birds housed on wet compared with those housed on dry litter. The higher expression of IL-1ß, IL-6 and IL-8 is consistent with an inflammatory response. From these data, plus results from Chapter 3, Experiment 3 showing that different litter types showed similar levels of FPD, it can be concluded that the reaction occurring in the birds as a result of FPD is an inflammatory one, with no definite evidence of a specific cell-mediated allergic response to an environmental factor.

No previously reported experiments had examined the effects of clean wet litter on FPD, researchers had simply applied extra water onto already dirty litter (such as Martland's papers from 1984 and 1985). The experiments reported here demonstrate that it is wetness rather than excreta that cause FPD. Several experiments carried out as part of the work reported here examined litter quality and type, and illustrated the fact that litter wetness, rather than the type of litter had more of an effect on FPD (Chapter 3). These findings supported previous work carried out by others (Harms & Simpson 1975; Harms *et al.* 1977; Harms & Simpson 1977; Martland 1985; Bray 1985; Bray & Lynn 1986; McIlroy *et al.* 1987).

The experiments described in this thesis indicate that a greater number of birds developed severe foot pad lesions when housed on wet litter, regardless of litter type, when compared with birds housed on dry litter. Different litter types may absorb

greater volumes of moisture, so the litter that absorbs the greatest volume of water without exposing the feet to wet litter may be the most effective at reducing FPD. Although no absorbency experiments were carried out as part of this project, it was observed that the recycled paper (Envirobed) and cardboard litters (BedXcel) absorbed a greater volume of liquid than woodchip or straw. Another method of dealing with excess litter wetness is the use of 'Mistral' (manufactured by Olmix, France), a commercial litter conditioner that can be applied to litter to absorb its own weight in liquid, thereby drying litter and reducing ammonia levels (Lumb 2002). (However, this compound was not examined as part of the experiments in this thesis).

Straw was used as a litter substrate, and even when dry, birds housed on straw developed more severe lesions than birds housed on other litter types. These findings supported previous work by Ekstrand & Algers (1997). This increase in FPD lesions from birds housed on straw may be due to the fact that straw does not absorb much liquid, resulting in areas of liquid from spilled drinkers or faeces amidst areas of straw.

It was postulated that there was a water-soluble irritant being released from the litter, probably originating from the wood, since birds housed on the three types of wood-based litter exhibited severe lesions. The litter types tested were woodchip, recycled paper, recycled cardboard and straw. The woodchip used in all the experiments was standard woodchip supplied to the farm units at the Roslin Institute, Edinburgh by Alex Anderson Farms (Bo'ness, UK). The woodchip was a mixture of redwood and

whitewood, largely pine, and was not chemically treated. The wood species present in the woodchip were identified as Scots pine and Spruce by the suppliers. An investigation of the soluble components of woodchip was undertaken contacting several forestry organisations and university departments as well as consulting several publications. Very little information was obtained regarding any soluble compounds that could cause adverse skin reactions when litter became wet. One possibility is the water-soluble compound, formic acid, found in red and white wood, and also used in paper processing. Scant information about wood chip production in the UK was available since it is mainly produced as a by-product of other processes. Chronic formic acid exposure may cause skin allergy in humans (www.answers.com)[5]. It is possible that chronic exposure to formic acid may also result in a skin reaction such as FPD in other species such as turkeys, if exposed to formic acid 24 h a day over several weeks.

Recycled paper and card processing are harsh processes, involving high temperatures and chemical treatments that could result in harmful residues. It is possible that water may act to release a harmful substance from a previously innocuous one, when it comes into contact with the woodchip or other processed wood litter.

Previously reported data (Lerner 1996; Lumb 2002) suggested that ammonia present in litter causes burn-like lesions that appear similar to FPD, and that may in fact be the same condition. However, Wang *et al.* (1998), disputed these findings, reporting that there was no difference in ammonia levels or litter pH between wet and dry litter

[5] Information gathered from the website ***www.answers.com/topic/formic-acid***

in hen housing. The results of the experiments carried out as part of this PhD (Chapter 3, Experiment 3) support the findings of Wang *et al.* (1998), since ammonia measurements were very similar between different litter types and on wet and dry litter after 6 days of the experimental period.

There is a possibility that the skin of birds affected by FPD have less collagen present within the skin, resulting in weaker skin of the foot pad. The skin strength of birds affected and those that did not become affected by FPD was tested using a Lloyd Instruments LRX50 Materials Tester (Lloyd Instruments Ltd, Hampshire, UK). Skin was taken from the foot pads of turkeys exhibiting FPD lesions as well as those unaffected by FPD. The skin was tested for shear and tensile strength. In birds with foot pad lesions, the skin always broke at the point of the lesion presumably because this was weaker than unaffected skin. The aim was to test the strength of the surrounding skin that was not displaying any effects of lesions, both in birds with and without lesions, to see if birds that developed lesions had weaker foot pad skin. However it was difficult to find any area of the foot pad that was not affected by FPD in older birds, as the lesions spread across the foot pad. Halliwell (1975) reported that avians had little protective fat and connective tissue directly under the metatarsal pad, leaving the footpad prone to mechanical damage. Weaker skin may result in an increased likelihood of developing FPD, as it would be more prone to mechanical damage. Unfortunately this experiment yielded inconclusive results, but it would be an interesting aspect to investigate in future.

Chapter 4 examined the effects of dietary biotin on the development of FPD. It had been reported previously that high biotin diets could prevent FPD (Buda 2000a, Buda 2000b; Wakeman 2000). On this basis, the experiments reported here tested varying levels of biotin, from 0-1600 μg/kg of feed in birds from hatch to 14 weeks. Food intake and weight gains were similar within all dietary groups, and no differences were observed between the severity or frequency of FPD in birds consuming different levels of biotin. It was concluded that biotin did not affect the development of turkey FPD.

An hypothesis had been put forward that increased pressure on the foot pad may increase the likelihood of developing FPD. Commercial turkeys in general have been bred to be larger than the wild type (Wylie 1999). Modern turkeys are less active than traditional turkeys, spending a greater amount of time sitting, thereby increasing pressure on the breast, hock and foot pads (Wylie 1999). Several experiments were conducted to increase the pressure on the foot pad of turkeys by fixing a small block of inert substance such as wood, plastic or metal to the foot pad with various glues. The most successful adhesive was Vetbond™ (3M Animal Care Products, Minneapolis, USA). However, after 24 h, the birds had pecked the blocks and removed them from their foot pads. It was concluded that this method of increasing and measuring foot pad pressure was not practical.

Turkey FPD may have a similar aetiology to human bedsores. Human pressure sores have been reported to occur as a result of one or a more of the following forces, pressure, friction and shearing (Simpson *et al.* 1996). Moisture due to sweat, urine

or faeces also increased the probability of developing pressure sores, since the skin became softened and abrasion of the skin was more likely (Torrance 1983). Data reported indicated that some people were more susceptible than others to developing sores. These individuals were those that may have been nutritionally challenged, immobile, had particularly low or high body fat (patients with a low body fat percentage showed increased pressure on bony areas, high body fat patients were more prone to friction and shearing forces when being moved), and incontinence (Torrance 1983).

The intensity and duration of the pressure applied to the skin determined the degree of tissue damage that subsequently occurred (Simpson *et al.* 1996). A document formulated for usage by the Edinburgh Healthcare NHS Trust (Steven *et al.* 1997) reported a scoring system used by healthcare professionals to classify pressure sores. Classification started with a reddening of the skin, which blanched when pressed (score 0). A score II was recorded when some skin thickness, either epidermis or dermis, had been lost, resulting in an abrasion or shallow crater. The most severe classification was a score IV in which there was extensive skin loss, tissue necrosis and damage to surrounding structures, and a 'crater' formed in place of the tissue. This classification of pressure sores was very similar to the external scoring system developed for examining turkey foot pads as part of this PhD. Damage to skin occurred in both human beings and turkeys in the same manner, beginning with reddening of the skin, followed by damage to the epidermis and dermis, necrosis, and extensive destruction of tissue as the lesions spreads. Moisture was found to be involved in the development of human pressure as well as in turkey FPD. Both

conditions result in localised inflammation and necrosis of tissue, but as yet there is no further knowledge of the similarity of the conditions.

From a welfare point of view, foot pad lesions have not been reported to be painful. Since FPD developed bi-laterally, it would be difficult to test if the condition was in fact painful, as the affected turkeys do not limp. A pedobarograph could be employed to test pressure applied to each area of the foot pad as well as to characterise locomotion (Corr *et al.* 1998). Foot pain did not cause the turkeys to cease to feed, as food consumption and weight gain was within normal limits for birds that did and did not develop lesions (see Chapter 4). An hypothesis can be formulated that FPD may decrease industrial production and profits, since carcasses would be downgraded if foot pads became so painful that the birds sat still for longer periods of time. Birds spending longer sitting in one position may suffer an increased frequency and severity of hock burn, breast blisters and breast buttons (Wylie 1999). However, if this condition is in fact painful, it should be managed and resolved to improve the welfare of the birds, even if it has no effects on profitability.

Overall, FPD has been found to be a condition that has been observed in poults as young as 3 weeks old in a commercial situation, and could be induced in young turkeys (4 weeks old) by housing on wet litter for 48 h. It was unimportant what litter type is used. Turkeys housed on wet litter developed severe foot pad lesions (indicated by inflammation and necrosis) within 48 h of being housed in such conditions. Turkeys then placed onto dry litter showed signs of skin healing in a short period of time, and within 15 d the microscopic cellular abnormalities present

were minimal. Expression of pro-inflammatory cytokines such as IL-1ß, IL-6 and IL-8 were upregulated in birds affected by FPD, indicating an inflammatory response. FPD may have resulted primarily from wet litter, but individual susceptibility may also play a part. Litter should ideally be better managed, that is, more frequently changed, possibly more absorbent litter should be used, more effective ventilation and heating systems employed to maintain dry litter, and the reduction of wetness in the first instance should be a priority. Drinkers should be designed and managed to minimise the amount of water spilled to reduce litter wetness.

Future work

Wetness has been shown to be a primary factor in the development of turkey FPD. Different types of litter should be examined and the amount of liquid they are able to absorb should be determined, as high absorbency materials used in turkey production may reduce FPD.

Formic acid is a possible irritant from the litter. It would be useful to study the effects of formic acid on the development of FPD by using a litter known to be free of formic acid, possibly by using an inert substance like sand as a control litter, then applying high levels of formic acid to a different group of pens. Litter ammonia is not likely to be affecting FPD, but further experiments to record litter ammonia levels from different types of litter would help to confirm findings that ammonia is not involved in the development of FPD.

No data is currently available regarding pain associated with FPD. It may be possible to test the degree of pain experienced by turkeys with FPD by training the birds to walk across a pedobarograph, then inducing lesions. Birds would then walk across the pedobarograph with and without an injection of a local anaesthetic. The walking patterns of the birds could then be compared. Another method may be to examine video footage of behaviour time budgets in birds with and without FPD to see if those affected spent more time sitting or shifting position.

Skin strength measurements were unsuccessful since skin broke at the point of the lesion. Since collagen is involved in skin strength, an investigation into the amount of collagen present in the foot pad skin of birds that are affected and unaffected by FPD, or in birds on wet and dry litter for a short length of time, for example, 24 h would answer the question as to whether birds that develop FPD have less collagen initially within the skin of the foot pad, resulting in weaker foot skin.

A larger scale repeat of the immmunohistochemical procedures reported in Chapter 5 would be useful, accompanied with both positive and negative controls would be useful to confirm that staining procedures had been successful as well as confirming that the cells observed were consistent with a simple non specific inflammatory response.

It would also be interesting to examine the development of both human pressure sores and FPD and assess how similar they are. Necrotic tissue from pressure sores and FPD lesions could be extracted for analysis using qRT-PCR to identify cytokine expression. If these two conditions were found to be similar, FPD might be a useful model for human bedsores.

Appendix 1: A record of Food Pad Dermatitis in the UK turkey industry

A major turkey producer in the UK supplied field data collected from a number of its farms over several years that were required by a major retailer. These records provide details of the farm site, bird sex, age in weeks, severity of the lesions (score 0, 1 are classified as mild, whilst 2 or 3 are classified as severe), and the date of culling. No descriptions were available for how foot pads were categorised or how foot pad scoring was carried out. Although these data do not specify enough information to be certain about any conclusions, it does allow for a suggestion of the trends within UK turkey production.

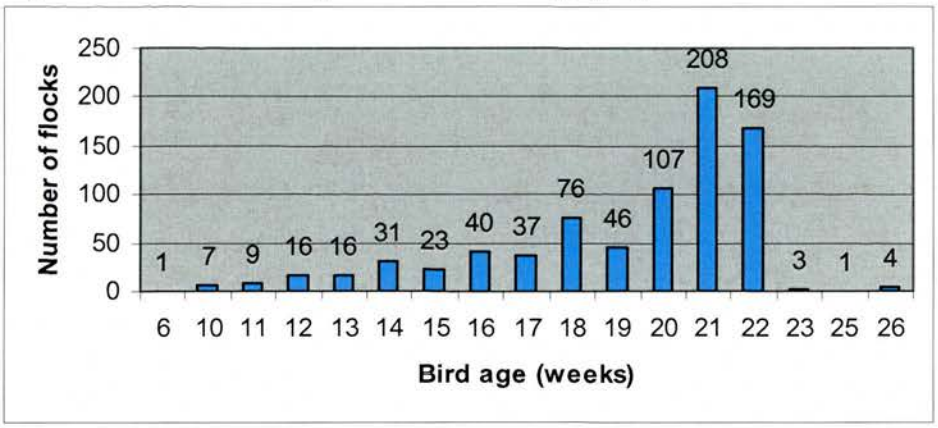
There were 676 flocks examined, from 74 farms, each sample was of either 100, 200 or 300 birds. The majority of sample sizes were of 200 turkeys.

Generally, the percentage of birds with foot pad lesions increased with bird age. Incidence of lesions was recorded at 56 % at 10 weeks, rising gradually until 100 % were affected at 26 weeks. Within those birds affected by FPD, the number of birds with severe lesions increased with bird age.

Table A.1. Association of bird age at point of killing with the mean percentage of birds showing any evidence of foot pad lesions, plus the percentage of mild and severe lesions within those affected.

Bird age (weeks)	No. of flocks within each age groups	Mean % of birds with lesions	Mild lesions, %	Severe lesions, %
10	7	56.4	94.6	5.4
11	9	53.5	93.2	6.8
12	16	67.7	88.6	11.4
13	16	80.0	88.4	11.6
14	31	86.2	79.2	20.8
15	23	87.3	80.0	20.0
16	40	83.0	81.9	18.1
17	37	88.9	78.3	21.7
18	76	86.4	76.7	23.4
19	46	84.8	71.9	28.2
20	107	95.5	62.6	37.4
21	208	92.8	61.2	38.8
22	169	90.1	66.1	33.9
23	3	94.3	62.0	38.0
25	1	99.0	29.0	71.0
26	4	100.0	30.5	69.5
All	676	88.9	69.2	30.8

Figure A.1. Number of turkey flocks within each age group.



There is a high incidence of FPD within both males and female turkeys. It appears that more stags show evidence of FPD than hens.

The data in Table A.2. show that hens have a higher percentage of mild lesions, and a lower percentage of severe lesions when compared with stags. However, hens are usually killed at a younger age stags, so time may be involved with lower FPD scores.

Table A.2. Bird sex with the mean percentage of birds showing mild and severe lesions.

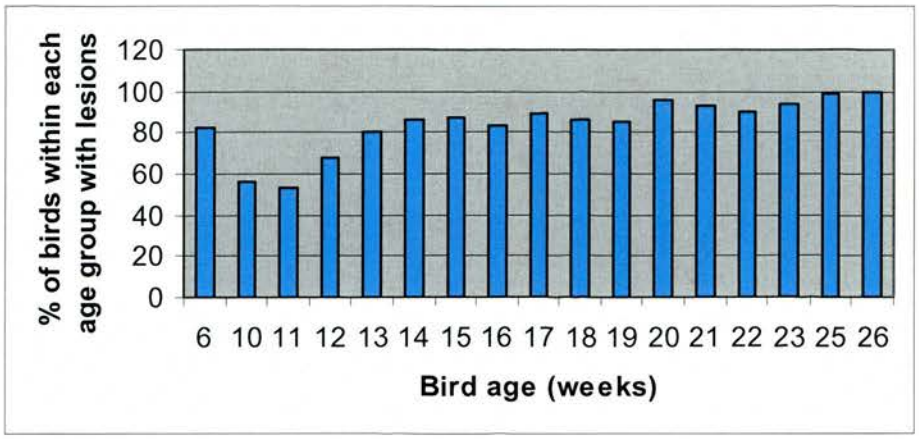
Bird Sex	Mean % of birds with mild lesions	Mean % of birds with severe lesions
Hens	81.3	18.8
Stags	63.3	36.8
All	69.2	30.8

Stags show evidence of FPD at an earlier age than hens (Table A.3.). At 14 weeks, stags show 91.82 % incidence of FPD, whilst hens at 14 weeks show only 82.66 %. The prevalence of FPD is consistently higher in stags than it is in hens. Overall, the total number of birds affected by FPD rose with bird age (Fig A.2.).

Table A.3. The percentage of birds showing evidence of FPD correlated with bird age and sex.

Bird age (weeks)	No. of flocks within each age groups	Mean % of hens with lesions	Mean % of stags with lesions	Mean % of all birds with lesions
10	7	56.4	-	56.4
11	9	53.5	-	53.5
12	16	67.7	-	67.7
13	16	80.0	-	80.0
14	31	82.7	91.8	86.2
15	23	88.0	85.6	87.3
16	40	83.0	-	83.0
17	37	88.8	91.3	88.9
18	76	85.5	92.5	86.4
19	46	82.4	90.8	84.8
20	107	87.0	95.6	95.5
21	208	78.5	93.0	92.8
22	169	91.5	90.1	90.1
23	3	-	94.3	94.3
25	1	-	99.0	99.0
26	4	-	100.0	100.0
All	676	81.8	92.5	88.9

Figure A.2. Percentage of birds affected with lesions within each age group

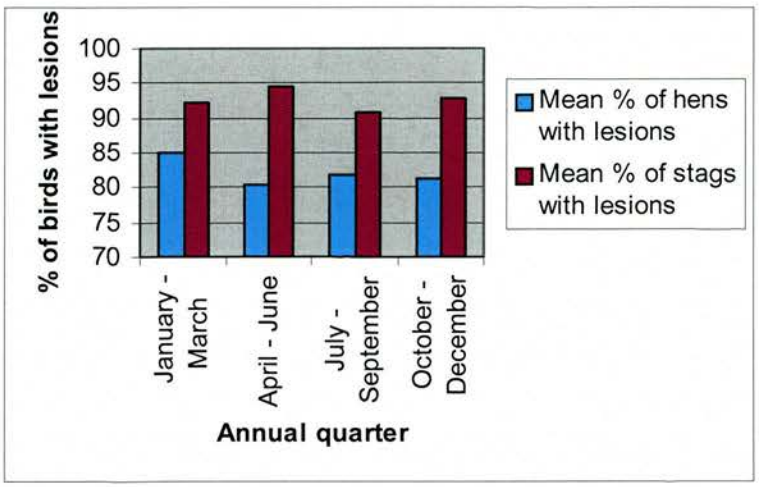


Stags showed an increased total incidence of FPD throughout the year when compared with hens (Table A.4. and Fig A.3.). The highest incidence occurred during the period of April to June (94.6 %), in stags. In hens the highest incidence of FPD occurred during the period of January to March (85.0 %).

Table A.4. The effects of season and bird sex on the total mean percentage of birds showing any evidence of foot pad lesions

Annual quarter	Mean % of hens with lesions	Mean % of stags with lesions	Mean % of all birds with lesions
January - March	85.0	92.2	90.6
April - June	80.5	94.6	89.4
July - September	81.7	90.8	88.2
October - December	81.3	92.9	88.0
All	81.9	92.5	89.0

Figure A.3. Percentage of hens and stags with lesions within each annual quarter



In summary, this survey highlights the fact that the incidence of FPD does increase with bird age. The percentage of mild lesions falls as the birds age, and the percentage of severe lesions rises with age.

The differences between the sexes are as follows. More stags show evidence of FPD than hens, with more stags showing evidence of FPD at an earlier age than hens. Hens have a higher percentage of mild lesions, and a lower percentage of severe FPD lesions than stags. The prevalence of FPD is consistently higher in stags than it is in hens.

These data provide an overview of the incidence of FPD at different slaughter ages. It would have be interesting to analyse these results statistically, but there were a large number of factor that were not consistent throughout the data collection period, which would not have allowed for a fair comparison of results.

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A review of the aetiology and possible causative factors of foot pad dermatitis in growing turkeys and broilers

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FPD is a common condition amongst commercially grown turkey poults. It causes the skin of the footpad to become hard and scaly, often developing horn-like pegs of abnormal keratin. The footpad can become swollen, frequently splitting. In the centre of the lesion the epidermis separates, and is often totally necrotic. Heterophils filter into the stratum germinativum.

The cause of FPD is complex, but many contributing factors have been suggested, such as dietary intake, skin structure, bird weight and sex, litter moisture and litter type. Litter quality is affected by many other factors such as stocking density, air temperature and moisture, season, consistency and amount of faeces (affected by diet), and drinker design.

Wet litter is the most likely factor affecting FPD, followed by biotin deficiency. Experimental evidence suggests that biotin deficiency causes FPD, and that commercial rations do not contain enough biotin to prevent these lesions. Supplementations of biotin have been shown to reduce the severity and incidence of lesions. Wet litter has also been identified as a possible causative agent. Broilers and poults reared on wet litter have an increased incidence and severity of FPD lesions, but the problem is alleviated by replacing the wet litter with dry. Biotin supplementations are able to reduce FPD to a certain extent if birds are reared on dry litter, but if on wet litter, lesions may still occur. Biotin intake and wet litter appear to be the factors most likely to influence the development of FPD.

Experimental results are difficult to compare because rearing conditions differ. Further experimentation is needed to determine the optimum amount of biotin required for healthy growth and lesion free foot pads, and to ascertain the real effects of other suggested causes.

Keywords: turkeys; foot pad dermatitis; pododermatitis; litter; biotin

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Introduction

The prevalence of footpad dermatitis (FPD) in turkeys is extremely high (Berg, 1998; Ekstrand *et al.*, 1997). Although there are various estimates of its prevalence, it is difficult to compare findings because the scoring systems used in different experiments are not the same. Paulus (2002) reported a prevalence of 70% among hens, and 78% among stags in the USA, whilst the UK showed 67% prevalence in hens, and 83% in stags. In a survey carried out by Ekstrand and Algers (1997) 98% of Swedish turkey poults had evidence of FPD. Berg (1998) estimated the prevalence of FPD in Swedish turkeys to be 20% for severe lesions (ulcers) and 78% for mild lesions (discolouration, erosion).

Several scoring systems for FPD are in use, one of these is the system based on the work by Martland (1984) in turkeys (*Table 1*). The same scale was later used to score broiler chickens for FPD (Martland, 1985). Both hens and stags were scored on the same scale, but since stags have been observed to have more severe lesions, the score of 3 was used to score stags only. Clark (2002) noted another scoring method as well as Martland's that is used by Poultry Intellimetrics, Inc, USA; both methods categorise lesions in a similar way. Commercial retailers and turkey production companies have their own visual comparison chart based on Martland's original scoring system.

Table 1 Martland's UK scoring system for classifying foot pad lesions (Martland, 1984; Martland, 1985).

Score	Lesion
0	None
1	Small scab(s) <5% pad area
2	Larger scabs <25% pad area
3 (stags only)	Severe, large scab-filled ulcers

External description of the condition

Poultry are often observed to have discoloured areas of skin on the foot, and slight lesions or ulcers on the footpad. Foot pad lesions have been reported to appear similar in both turkeys and broilers, although no work has compared the lesions in both turkeys and broilers (Bruce *et al.*, 1990; Ekstrand *et al.*, 1997; Ekstrand *et al.*, 1998; Ekstrand and Carpenter, 1998a; Ekstrand and Carpenter, 1998b; Ekstrand and Carpenter, 1998c; Harms *et al.*, 1977; Martland, 1985; Patrick *et al.*, 1942; Scott, 1981).

Lesions begin as small scaly brown scabs on the surface of the metatarsal and digital pads, becoming cracked and eroded and progressively larger over the first few weeks of life along with acute inflammation, swelling, hyperplasia and necrosis of the epidermis (Greene *et al.*, 1985; Pass, 1989). Plant material and bacteria were often present on the surface within necrotic debris, but were seldom found in deeper layers (Greene *et al.*, 1985; Martland, 1985).

Histopathological changes that occur within the foot pad

Platt *et al.* (2001) studied the development of foot lesions in turkey poults. At 6 weeks they observed hyperkeratosis of the footpads plus separation of keratin layers. Lesions were mostly superficial but sometimes ulceration spread into the dermis. The number of lymphocytes, granulocytes, and lymph follicles increased within the dermis adjacent to the lesions. After 14 weeks, the prevalence of superficial lesions decreased, whilst more severe ulceration increased.

The mildest lesions showed an infiltration of heterophils into the stratum germinativum, and defects in keratin formation (Martland, 1984). Greene *et al.* (1985) also noted heterophils in the dermis, sub-epidermis, and epidermis as well as basophilic debris (necrotic cells) in the stratum corneum. Small vacuoles (often containing heterophils) were seen in the epidermis and inside blood vessels (Greene *et al.*, 1985; Harms and Simpson, 1975; Martland, 1984; Martland, 1985). In the centre of the lesion, there was complete destruction of the keratin and epidermal layer, exposing necrotic tissue and a mass of inflammatory cells, predominantly heterophils (Greene *et al.*, 1985).

In more severe, ulcerated lesions, all the above observations were evident, but the major finding was acute inflammation. More dense cellular infiltration occurred, and there were more obvious defects in the stratum corneum such as thickening and the formation of 'horned pegs' (Martland, 1984; Whitehead, 1990). The epidermis had become more eroded, and fluid filled the dermis. Blood vessels were congested and dilated, and sometimes necrotic (Whitehead, 1990).

Foot lesions observed in older turkeys appeared to be different from those seen in younger birds. Younger birds show scaliness and/or thickening and cracking of the skin, whilst older birds also exhibit an enlarged ball of flesh within the footpad that sometimes included scar tissue (Richardson and Wilgus, 1967).

Possible causes of FPD

The cause of FPD is complex. Many factors (that will be reviewed below) have been suggested, but it is more likely that the cause is multifactorial. Ekstrand has published a number of papers highlighting the rearing conditions, and prevalence of FPD in both broilers and turkeys in Sweden. She hypothesises that FPD is a contact dermatitis that is caused by wet litter (associated with drinker type), litter depth, and litter material.

FPD has been associated with the prevalence of breast lesions (Gonder and Barnes, 1987; Harms and Simpson, 1975; Kamyab, 2001) and hock burns (Bruce *et al.*, 1990). From reports of breast, hock and footpad lesions, the pathology of each appears similar to a contact dermatitis (Gonder and Barnes, 1987; Greene *et al.*, 1985; Martland, 1984). Breast lesions showed a central scab composed of exudated protein and necrotic inflammatory cells. This was sometimes covered by cornified strands of keratin. This description is similar to lesions seen on the footpad.

Internal factors

SEX

Female skin contains more fat, and less protein and collagen than males. This suggests that female skin may be more likely to tear than male skin, as the protein matrix will be less dense and therefore easier to pull apart (Kamyab, 2001). Halliwell (1975) stated that birds of both sexes had little protective fat and connective tissue directly under the metatarsal pad, leaving the footpad prone to mechanical damage.

There is a higher incidence of lesions in males compared with females in some reports (Buffington *et al.*, 1975; Harms *et al.*, 1977; Harms and Simpson, 1975; Harms and Simpson, 1977; McIlroy *et al.*, 1987). However, other work has reported no significant difference between the prevalence of FPD in males and females (Ekstrand and Algers, 1997; Martland, 1984).

BREED

Large White turkey poults were found to be more susceptible to FPD than Broad Breasted Bronze poults when reared in the same conditions on wire floors. Using a scoring system of 0 for a normal foot pad, and 4 for a severe lesion, Large White poults had an average score of 0.60, whilst Bronze poults had a score of 0.20 (Chavez and Kratzer, 1972). However, this may be due to the fact that Large White poults had a more rapid rate of growth than Bronze poults, resulting in heavier birds.

DIET

Vitamins and amino acids such as biotin, pantothenic acid, methionine, zinc, lysine, riboflavin (also known as vitamin G and flavin) have all been reported to be involved in skin formation and maintenance (*Table 2*). Deficiency symptoms such as FPD result if an inadequate amount of these vitamins and amino acids are consumed. Chavez and Kratzer (1972; 1974) reported that a methionine deficiency resulted in a higher prevalence of FPD in White turkey poults. Other dietary factors may also affect FPD, such as a high soybean content, and soybean meal replacements (SBM). The former may cause FPD due to its low natural biotin content, and the latter as it contains indigestible fats, increasing litter wetness and causing litter and faeces to adhere to the foot pad, possibly causing a lesion (see *Table 2*). Harms and Simpson (1982) reported that higher levels of dietary NaCl, increased the rate of growth of turkey poults, which in turn increased the severity of FPD recorded in these birds.

Table 2 Dietary factors (apart from biotin) that have been reported to affect foot pad dermatitis.

Dietary factor	Reported by	Experimental results
Methionine	Chavez and Kratzer, 1972; Chavez and Kratzer, 1974; Murillo and Jensen, 1976	Methionine dietary supplementations significantly reduced FPD incidence
Pantothenic acid	Kratzer and Williams, 1948	Pantothenic acid deficiency results in FPD and lesions on the eye and mouth
Riboflavin	Lepkovsky and Jukes, 1936a; Lepkovsky and Jukes, 1936b	Riboflavin is needed at a level of 2700 µg/kg during first 6 weeks of life, or severe FPD results
Zinc	Whitehead, 1990	Zinc deficiency results in FPD lesions
Zinc plus amino acid complex	Hess *et al.* 2001	FPD lesions were significantly reduced in birds fed a zinc and amino acid complex
Soybean meal	Abbott *et al.*, 1969; Jensen *et al.*, 1970	Birds fed a soybean meal diet developed a high incidence of FPD possibly due to the low level of naturally occurring biotin
Soybean meal replacements (SBM)	Boling and Firman, 1967; Leeson and Summers, 1991; Murakami *et al.*, 2000	SBM contain 'bad fats' (oligosaccharides) that are problematic to digest leading to watery and sticky droppings. This may increase the probability of faeces and litter adhering to the birds feet and causing FPD lesions

The role of biotin in avian species

Biotin is required in carbohydrate metabolism, fatty acid synthesis, protein synthesis (through its effects on ribonucleic acid formation), amino acid synthesis, amino acid deamination, purine synthesis, and nucleic acid metabolism formation (Whitehead, 1977). Because biotin is needed for the vital basic functions, it has an effect on most major systems, especially the cutaneous system.

Biotin deficiency symptoms

Biotin deficiency results in a hard scaly dermatitis that appears first on the foot pads of turkeys and broilers (Greene *et al.*, 1985; Oloyo, 1991; Patrick *et al.*, 1942; Whitehead, 1977; Whitehead and Bannister, 1981). Biotin deficiency may also reduce growth and feed efficiency. Turkeys are particularly at risk from biotin deficiency symptoms, as they have the highest biotin requirement of any bird species investigated so far (Scott, 1981; Whitehead, 1990). Biotin deficiency is often identified in growing birds by a number of external symptoms (*Table 3*). Internal cellular changes that can only be observed with the use of a microscope also take place as a result of biotin deficiency (as summarised in *Table 4*).

Experimental evidence for recommended biotin concentrations

Commercial rations (containing 200-300 µg/kg biotin) have been claimed to contain an adequate amount of biotin to prevent FPD in poults. Poults did not show biotin deficiency

Table 3 External biotin deficiency symptoms in growing birds.

Biotin deficiency symptom	Reported by
Depressed growth	Patrick *et al.* 1942
Poor food conversion rate	Oloyo, 1991; Patrick *et al.* 1942
Leg abnormalities (metatarsal bones become shortened and the hock joint becomes distorted which may lead to perosis)	Oloyo, 1991; Patrick *et al.* 1942; Whitehead, 1990; Whitehead and Bannister, 1981
Lesions on the eyelids, beak, and vent	Whitehead, 1990; Whitehead and Bannister, 1981
Lesions of the foot pad including ulceration and cracking of the foot pad	Oloyo, 1991; Patrick *et al.* 1942; Whitehead and Bannister, 1981; Whitehead, 1990
Increased mortality	Oloyo, 1991; Patrick *et al.* 1942

Table 4 Internal biotin deficiency symptoms in growing birds.

Biotin deficiency symptom	Reported by
Increased liver and kidney weights	Oloyo, 1991
Increased lipid deposition within the liver and kidney	Oloyo, 1991
Raised blood lipid levels	Oloyo, 1991
Lower blood glucose levels	Oloyo, 1991
Lower pyruvate carboxylase activity in the liver	Oloyo, 1991
Acanthosis within the integument	Harms and Simpson, 1982; Oloyo, 1991
Hyperkeratinisation within the integument	Harms and Simpson, 1982; Oloyo, 1991
Abnormal keratinisation and cornification of the epidermis	Buda, 2000a; Buda, 2000b

symptoms if the feed contained 250-255 µg biotin. It has been concluded that commercial turkey rations contained adequate biotin, as only a very low level of FPD was observed. However, it was commented that biotin supplementation was effective in reducing, although not completely eliminating FPD (Robblee and Clandinin, 1970; Sullivan and Platter, 1969). Contrary to these findings, others have observed symptoms of biotin deficiency, such as lesions of the eye, vent, beak, and feet when poults were fed commercial rations without supplementation. Symptoms have been reduced in severity by administering biotin supplementation.

Richardson and Wilgus (1967) reduced the severity and incidence of FPD by adding 250 µg biotin per gallon of water to the drinking water of poults from 9 days old.

A biotin supplement of 135 µg/kg did not reduce the frequency or severity of FPD in turkeys (Johnson, 1967). However, with a supplementation of 250 µg/kg, the poults showed a near total recovery. Older stags (14 weeks) showed no recovery from the condition when given 500 µg/kg orally for 5 weeks, or 22 week old stags injected with 1000 µg for the first week, followed by 500 µg weekly. The lesions persisted despite the high levels of biotin provided. A possible explanation for these results is that FPD is preventable in its early stages by increasing biotin supplementations in young poults, but once the condition has become more severe, it is harder to rectify the situation.

Jensen *et al.* (1970) fed poults a supplement of 250 µg biotin/kg resulting in a 0% incidence of FPD at 2, 4 and 8 weeks. However, when the diet was high in soybean, the same amount of biotin did not reduce FPD. Other poults given a diet containing only 50 µg/kg had over 50% FPD at 4 weeks.

Marusich *et al.* (1970) showed that turkeys on a diet of 100 µg/kg had a 98% incidence of FPD that dropped to 0% above 225 µg/kg supplementation. Turkeys given 250 µg/kg in their feed showed a 53% incidence of FPD when no supplementation was given, falling to 5% when provided with 400 µg/kg supplementation. Misir and Blair (1988) and Platt *et al.* (2001) found similar results to Marusich *et al.* (1970). In contrast, Whitehead (1977) claimed that just 300 µg/kg was adequate to prevent FPD in growing turkeys.

Some more up to date experiments have demonstrated that biotin supplementation is still required to minimise FPD as commercial diets appear not to contain adequate biotin to prevent FPD. Recent studies have found that 2000 µg/kg given to stags between 9-20 weeks reduced FPD dramatically (Buda, 2000a; 2000b). In these experiments, commercial diets containing an average of 300 µg/kg of biotin were found to result in FPD, so a large supplementation (2000 µg/kg) was provided to remedy this. The biotin treated group had a lower FPD score (1.42) than the control group (1.72) on the Martland scoring system. No other levels of supplementation were reported to have been tested, and it may be that a lower level may suffice to prevent FPD as an optimum level was not defined.

Dietary factors that may affect biotin bioavailability

Biotin deficiency may be affected by other supplements. In growing chickens, biotin levels are depleted by supplements of high levels of choline and B-vitamin mixtures (Whitehead *et al.*, 1976; Whitehead and Randall, 1982).

Various drugs and antibiotics have been suggested to affect biotin availability (Waibel *et al.*, 1969). Slinger and Pepper (1954) suggested that the addition of an aureomycin-B_{12} supplement increased the availability of biotin within the feed, or possibly increased the synthesis of biotin within the intestines of the bird. This hypothesis was formulated because birds given the supplementation showed no signs of FPD or other symptoms of biotin deficiency. Of the birds on the control diet, 9% exhibited FPD after 8 weeks.

Male and female biotin requirements

There is evidence that males require a higher intake of biotin (50 µg/kg) than females to maintain optimum health (Dobson, 1970). Frigg (1984) found that males needed an extra 10 µg to maintain the same percentage of growth as females. This would explain why males are more likely to exhibit signs of deficiency (Harms *et al.*, 1977; Harms and Simpson, 1975; Harms and Simpson, 1977; McIlroy *et al.*, 1987).

PRESSURE AND BODY WEIGHT

Wylie (1999) suggested that the most important factor in causing breast lesions is thought to be pressure, followed by shearing forces, friction and moisture. These factors may also affect the footpads and cause similar lesions.

Commercial turkeys in general have been bred to be larger than the wild type (Wylie, 1999) and males are heavier than females. Modern turkeys are less active than traditional turkeys, spending a greater amount of time sitting, and so increasing pressure on the breast, hock and foot pads as turkeys sit resting on these areas (Wylie, 1999). When turkeys stand, which they must do to feed, the small area of the foot pad bears the full weight of the bird. As body weight increases, the force per area (pressure) of foot pad increases, and hence the pressure on the foot pads in heavier birds will increase. However, no work has reported if pressure on the foot pads increases with bird weight. Males are more likely to develop FPD lesions (Harms *et al.*, 1977; Harms and Simpson, 1975; Harms and Simpson, 1977; McIlroy *et al.*, 1987), which seems logical if pressure is a contributory factor in the cause of FPD. Some factors may reduce growth rates, and this may affect this issue of pressure on the foot pads. Biotin positively affects growth rate (Frigg, 1984), as does dietary NaCl (Harms and Simpson, 1982; Murakami *et al.*, 2000), and DL-Methionine and its precursors (Chavez and Kratzer, 1972; 1974). However, Buffington *et al.* (1975), and Martland *et al.* (1984), found no correlation between weight and the incidence or severity of foot and leg abnormalities in Wrolstad White turkeys.

External factors

Litter quality and type may be important in the prevalence of FPD as they are in contact with the footpad. Litter quality is affected by many variables, *e.g.* moisture levels may be affected by the type of drinkers provided, humidity, season, amount and consistency of faeces, and stocking density. The type of litter provided is also important, as different substrates may absorb varying amounts of liquid, and cause varying amounts of friction on the footpads of the birds.

Lesions develop at points of contact between skin and the ground and it is these weight bearing surfaces that undergo the cellular changes that characterise FPD (Whitehead, 1990). Therefore Greene *et al.* (1985) concluded that FPD was a contact dermatitis and suggested that poor litter conditions may be responsible.

WET LITTER

Foot, breast and hock lesions increased in severity when bird litter was wet. The severity of lesions increased in broilers and turkeys reared in pens containing wet and sticky litter (Harms *et al.*, 1977; Harms and Simpson, 1975; Harms and Simpson, 1977; Martland, 1985; McIlroy *et al.*, 1987). Growth rates were depressed within one week of being in a pen of wet litter. After 9 weeks of being reared on wet litter, the difference between weights of birds on wet and dry litter was highly significant. These birds were observed with severe skin ulceration on the plantar surface of the foot, the caudal aspect of the intertarsal joint and over the sternum. The prevalence of these alterations in skin

conditions was significantly different compared with birds raised on dry litter. By changing the wet litter for dry litter growth rate recovered (Martland, 1985), and lesions began to heal (Greene *et al.*, 1985; Martland, 1985). Turkeys reared on wet litter, especially if particularly deep, were found after 20 weeks, to have a larger mean number of lesions, when compared with those raised on dry litter. In turkeys that did have lesions, a larger mean percentage of the foot pad was ulcerated on the feet of birds raised on wet litter when compared with those reared on dry litter (Martland, 1984).

Housing birds on wet litter also increases the chance of faecal adhesion to the feet, which has been hypothesised to induce FPD (Jensen *et al.*, 1970). A mixture of faeces and litter sticks to the foot pad and dries on, becoming extremely solid. This may result in cellular changes within the epidermis simply from the presence of a foreign body stuck to the foot pad and increasing the pressure on the area, or from faeces irritants.

Wet litter was found to contain a higher concentration of nitrogen and a lower pH than dry litter (Lerner, 1996), resulting in higher concentrations of volatile ammonia within the litter. It is the ammonia in the top layer of litter that Lerner (1996) claimed causes focal ulcerative dermatitis (FUD) lesions, that have been linked with FPD (Kamyab, 2001). These data suggest that the effect of wet litter is stronger than the effect of increased body weight in regards to the development of FPD. Experimental evidence already reported suggests that wet litter is the most important factor affecting the development of FPD. Other factors such as dietary intake, breed, sex and bird weight may exert less of an effect.

Drinker design

Small cup drinker designs reduced litter wetness (compared with 11 other types of commercial drinkers), and decreased hock burn which is thought to be exacerbated by sitting in wet litter (Tucker and Walker, 1999). In support of this finding, Ekstrand and Algers (1997) reported that flocks with small cup drinkers showed a lower prevalence of FPD when compared with bell drinkers.

Diet and its effects on excreta

The moisture content of the litter will be affected by the consistency of the faeces and this in turn will be affected by the diet (Ekstrand and Carpenter, 1998c; Jensen *et al.*, 1970). Soybean meal contains fats which are difficult to digest (Boling and Firman, 1997; Murakami *et al.*, 2000). The fats in soybean meal cause reduced digestibility of the feed and may lead to watery faces and wet, greasy and sticky litter, increasing the probability of faecal material adhering to the birds' feet, and increasing the incidence of hock burn and FPD (Tucker and Walker, 1999). Litter wetness scores also increased significantly as dietary sodium levels increased (Murakami *et al.*, 2000).

Air temperature and its effect on litter moisture

If the temperature falls below a set point (the dew-point temperature), condensation occurs on exposed surfaces, including litter (Tucker and Walker, 1999). The risk of condensation is increased if the temperature is low, and relative humidity is high. Air temperature and humidity will be affected by stocking density, season and ventilation system (Alchalabi, 2002). Poor litter quality and poor ventilation may result in high levels of ammonia, carbon dioxide and hydrogen sulphide.

Lesions of the hock and breast in broilers were greatest during the winter months, when litter quality was at its worst. This may be related to the effects of relative humidity and outside air quality (Bruce *et al.*, 1990; Ekstrand and Algers, 1997; Ekstrand and Carpenter, 1998b; Ekstrand and Carpenter, 1998c; McIlroy *et al.*, 1987). There were significantly more recordings of poor litter quality (*i.e.* presence of wet and/or sticky litter) during the winter months (McIlroy *et al.*, 1987).

Stocking density

Lesions of the foot pad, breast and hock increased in broilers when stocking density increased (McIlroy *et al.*, 1987; Svedberg, 1988). This is probably related to poorer litter quality because there were more cases of poor litter quality in highly stocked pens (<0.48 sq ft/bird) when compared with low density pens (0.49 or more sq ft/bird).

LITTER TYPE AND FRICTION

Chen *et al.* (1991) stated that four types of slotted flooring (made of concrete, wood, fibreglass and PVC) had no significant effect on either the footpad score or prevalence of FPD in market turkeys.

The type of litter appears to have a marked effect on the incidence of FPD in turkeys (Hester *et al.*, 1997). Fine and coarse particleboard residues were compared with hardwood shavings as a control. It was found that turkeys reared on fine particleboard in Hester's experiments had a lower incidence of FPD, breast lesions and foot abnormalities, than coarse residue. Ekstrand and Algers (1997) found that poults reared on straw in commercial conditions showed a higher prevalence of FPD than those on wood shavings. However, McIlroy *et al.* (1987) and Bruce *et al.* (1990) found no significant difference in the occurrence of hock and breast lesions in broilers reared on straw or wood shavings in commercial flocks. Both types of litter were equally as likely to deteriorate in quality and become wet and sticky, and both led to the same incidence of lesions.

Discussion

Many factors have been suggested to cause FPD. The factor most likely to have an effect on FPD appears to be the presence of wet litter in bird houses, followed by dietary biotin. However, the condition is likely to be multifactorial, with other factors such as skin strength, bird weight, sex and breed also playing a part in the development of lesions. These secondary factors may have less of an effect than wet litter and biotin intake.

Biotin has been documented to be involved in skin formation and maintenance. Supplementations of between 250-2000 µg/kg have been found to reduce the incidence FPD. Biotin requirements may differ between experiments due to other factors not mentioned, such as the bioavailability of other nutrients in the diet, the wetness of the litter (which itself is affected by various other dietary ingredients), ventilation, humidity and temperature within the house. There may also be differences between turkey breeds used.

However, even in the presence of adequate biotin, wet litter is also known to cause FPD and may overcome the protective effects of having an adequate biotin intake (Platt *et al.*, 2001). Poor litter conditions may be the dominant factor in this equation. Since biotin is vital in skin maintenance, a lack of biotin does not allow for normal skin development and repair, meaning that as foot pads become eroded by wet litter and irritants such as ammonia and the resulting tissue damage is not as easily repaired.

Wet litter, from water in the drinkers and contained within the bird's faeces is associated with FPD. Many factors affect the moisture within the litter, including the design of the drinker; the diet consumed by the birds (as this influences the consistency of the faeces); air temperature and humidity (season has an effect on air temperature and humidity); high stocking density may lead to a higher rate of litter deterioration; and the type of bedding material may affect the amount of moisture that is trapped within the litter.

Wet litter is thought to have a higher concentration of nitrogen and a higher pH than dry litter, resulting in higher concentrations of volatile ammonia within the litter (Alchalabi, 2002). This has been hypothesised as a causative agent of FPD. However, it may be simply the wetness, as continually standing in moist conditions will soften the footpads and result

in the skin being more prone to mechanical damage. It is possible that poor skin strength may predispose birds to developing FPD.

Wet litter causes litter and faeces to adhere to the footpads and when dry the material becomes extremely hard. Irritation may occur, causing lesions as the result of increasing pressure on certain areas of the pad that may underlie the resulting cellular reactions.

It has been assumed that FPD is a welfare problem, with lesions causing pain to the bird and walking difficulties, but rarely causing lameness (Berg and Algers, 2004), although no work has provided evidence of this connection yet.

Further experimental work should be carried out to determine the mechanisms by which wet litter increases the prevalence of FPD and to determine the moisture content of litter above which turkeys and broilers are prone to develop FPD. Also, further work should ascertain the exact amount of biotin required by different breeds, and under different environmental conditions for the prevention of FPD. Biotin needs may be less if the birds are reared under good environmental conditions.

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Foot pad dermatitis develops at an early age in commercial turkeys

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Abstract 1. A field experiment was conducted to identify the macroscopic and histological changes associated with the development of foot pad dermatitis (FPD) in growing turkeys. Two affected and two unaffected turkeys were sampled weekly from 1 to 8 and at 10 and 21 weeks of age.

2. At one week old, birds with external signs of FPD (surface skin discolouration) showed abnormal cellular changes of the foot pad integument. As the flock aged the reactions intensified, with one sample exhibiting a fully developed macroscopic lesion at 3 weeks.

3. Major pathological changes had occurred by 6 weeks and all turkeys with external signs of lesions had fully developed microscopic inflammatory cellular lesions. From 6 weeks of age onwards lesions were increasingly numerous and became more overtly necrotic.

4. Externally normal foot pads showed microscopic evidence of lesions after the turkeys reached 4 weeks.

5. We conclude that FPD lesions become severe over a short period of time and at a very early age.

INTRODUCTION

Foot pad dermatitis (FPD) has been reported to be extremely common in commercially grown turkeys (Ekstrand *et al.*, 1997; Berg, 1998). Although it is widely accepted that the occurrence of FPD is high, there are few exact figures for its prevalence. Paulus (2002) claimed that the figure was between 67 and 83% in commercially grown males. Although the incidence is high, not all of these turkeys experience severe lesions and some may have mild superficial lesions affecting only the surface integument.

A scoring system for the severity of the lesions is important in determining the prevalence of the condition. A number of these exist, the major ones being those of Martland (1984, 1985) and Ekstrand *et al.* (1997). These scoring systems are largely chart based and allow the foot pads of live turkeys to be categorised before dissection, on the basis of their external appearance. These scoring systems assess the degree of lesion development according to the size of the lesions, and the extent to which the whole foot pad is necrotic. The problem with current scoring systems is that the early stages of FPD are not easily assessed. The scoring systems begin to record damage once the foot pad is already noticeably necrotic. Early stages of FPD such as subtle appearances in skin colour or texture are not included in current systems. Other scoring systems used by major turkey producers and supermarket companies are all based on modifications to these previously published systems. Since no histopathological scoring systems are currently available, the primary author of the present paper devised a scale to record cellular changes within the turkey foot pad (Table 1).

Previous work has examined the progression of FPD on the basis of external scores and showed that lesions begin as scaly skin on the foot pad, developing into cracks, with haemorrhaging and scabs on the metatarsal and digital pads (Whitehead, 1977; Greene *et al.*, 1985). Histopathological work has mostly examined the cellular changes present after lesions have occurred (Richardson and Wilgus, 1967; Harms and Simpson, 1975; Martland, 1984, 1985; Greene *et al.*, 1985; Gonder and Barnes, 1987; Whitehead, 1990). One recent study (Platt *et al.*, 2001) recorded the cellular changes in 6-week-old poults, and again at 14 weeks, but no previous study has examined the gradual weekly progression of FPD from the first week of life of the turkeys up to market slaughter age (at 21 to 22 weeks of age). Also, no previous work has taken

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Table 1. *A scoring system for categorising the histopathology of turkey foot pad dermatitis (devised by R.K. Mayne)*

Score	Description	Definition	Number of foot pads (left and right) recorded in each category in turkeys aged 1 to 10 weeks
0	None	No change, sample normal	13
1	Mild	Hyperkeratosis; 'Horned pegs' of keratin on surface; Epithelial hyperplasia; Compressed keratin on foot pad surface	11
2	Mild	Epidermal acanthosis; Increased dermal blood vessel density	9
3	Mild	Vacuoles in dermis/epidermis; Necrotic debris in keratin/epidermis	15
4	Medium	Presence of heterophils, macrophages and lymphocytes in dermis	9
5	Medium–severe	Increased density of heterophils, macrophages and lymphocytes; Congested/necrotic blood vessels; Necrotic debris of cells in dermis/epidermis	12
6	Severe	Split epidermis–1 lesion	9
7	Severe	Split epidermis–1 + lesion or 1 very large lesion, more than 1/3 of total sample	2

samples of externally normal foot pads for comparison with affected ones.

This experiment sampled turkeys from one week of age up to 21 weeks and compared apparently normal foot pads, that is, no external evidence of necrosis or reddening of the skin, with those foot pads exhibiting lesions. Although lesions have generally been associated with turkeys aged 8 to 12 weeks, it has been suggested that lesions can start at a very early age as a minor area of discolouration of the dermis. The major aim of the study was to evaluate this hypothesis and track the development of lesions at a histopathological level in commercially grown turkeys. Our ultimate aim is to identify potential mechanisms that may indicate the cause of FPD as a necessary step to preventing it occurring in commercial turkeys.

MATERIALS AND METHODS

Experimental design

Forty turkeys were sampled from 4 separate farm units, according to age, on the farms of one of the two major commercial turkey producers in the UK. Each farm site housed birds of a similar age; for example, one farm housed birds aged 1, 2 and 3 weeks. All turkeys were sampled from the different farm sites over two consecutive days to ensure there was no difference in results due to different sampling times. All turkeys were kept in identical houses, in identically laid out farm sites, all within a 15 mile radius of each other, all owned by the same company. Since the farms were all laid out in the same manner and had the same timetables and management systems, any effects from different farm sites should have been minimised. Four turkeys were randomly sampled from each age group; two turkeys with apparently normal foot pads (meaning that there were no external signs on the foot pads that FPD had occurred, or was beginning to occur) and two turkeys with obvious signs of foot pad lesions, that is, necrosis of the foot pad, or subtle changes that indicated lesions may be beginning, such as reddened or roughened skin. Both feet were taken from turkeys at 1, 2, 3, 4, 5, 6, 7, 8, 10 and 21 weeks of age.

Turkeys and housing

All turkeys sampled were Large White Broad Breasted turkeys, and all were male, except turkeys aged 4 weeks, which were female, as no males were available within that age group. However, since both male and female turkeys at this age are similar in size and level of development, no difference in foot pad status should have occurred due to sex.

All turkeys were fed *ad libitum* on a commercial pellet diet formulated from recommendations by the breeder. The turkeys were kept in large rectangular barns of several thousand turkeys, housed by age. All turkeys within one house were hatched at a similar time, within a few days of each other. Turkeys of different ages were housed in different houses within a relatively small area. All houses were ventilated and temperature-controlled according to recommendations for the specific age of the turkeys. The litter in each house was whitewood shavings kept at a depth of 10 to 15 cm. Litter quality was of a similar standard in all houses examined. Litter was clean but slightly damp in all houses to the extent that it left an imprint when walked upon.

Sample collection, preparation and scoring

Turkeys were collected by the farm staff and the first author, and the latter examined the turkeys on the farm site. The turkeys sampled were either found dead that morning, having died as a result of injury such as head pecking or broken wings, or were culled by cervical dislocation by the farm staff in the poultry houses that morning (no more than 2 h previously). No obviously diseased turkeys were used for sampling; culled turkeys were apparently normal in physical development, size and health, but had been damaged physically by vent or head pecking, broken wings or other physical injury. All turkeys used for sampling were therefore physically injured in some way, whether they were found dead, or culled as a result of serious physical injury. However, such physical damage should not affect foot pad status as these injuries were mechanical upper body injuries, and unrelated to skin lesions on foot pad. Several flocks of different ages were sampled at the same time on the same farm site. Injured turkeys were examined for signs of lesions on the foot pad. Since there were a number of injured turkeys per house, there were enough mortalities to be able to obtain two turkeys with signs of FPD and two without. Both feet from each turkey were photographed using a digital camera, and a brief description of the foot pads was recorded. The central metatarsal pads from both feet were then removed and placed in tubes of 10% buffered formalin.

Samples were transported to the Easter Bush Veterinary Centre (EBVC), Edinburgh. Slices were cut from each sample, processed as standard paraffin wax sections, stained with haematoxylin and eosin and examined by light microscopy. Foot pad slides were scored using histopathological criteria (Table 1).

RESULTS

Control turkeys

In very young turkeys (up to 4 weeks), the foot pads that appeared normal externally (Figure 1(a)), also appeared normal microscopically. At later ages (from 4 weeks), all foot pads that appeared normal upon first inspection showed evidence of minor cellular changes (classified as a mild lesion, Table 1), suggesting that a lesion was beginning to develop. Although 40 turkeys were sampled, and half of these showed no signs of external lesions, there were still histopathological changes occurring in some of these birds (mostly in older turkeys). The table shows these results, and shows that there are less than 40 foot pads (two from each turkey with externally normal foot pads) assigned a histopathological score of 0. However, the cellular changes within these foot pads were of a lesser degree than those found in turkeys with obvious macroscopic lesions. These minor changes (Figure 2) included abnormal keratin formation such as loosely packed but apparently normal

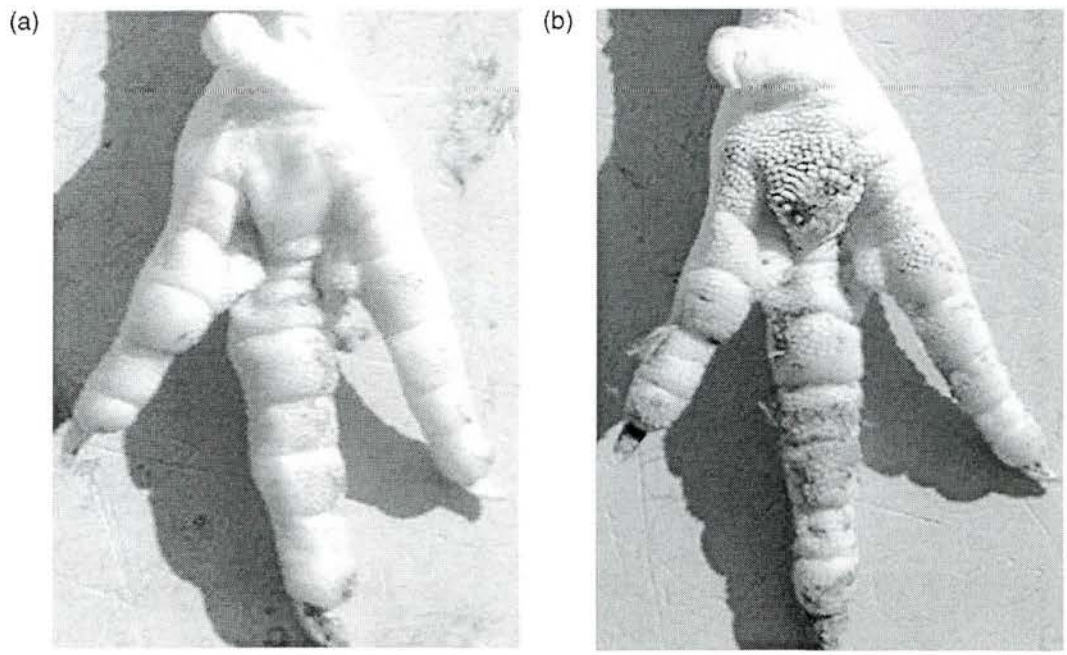

Figure 1. *External photograph of turkey foot pads: (a) normal foot pad in a 6-week-old turkey (external score 0); (b) foot pad affected by FPD in a 6-week-old turkey (external score 6).*

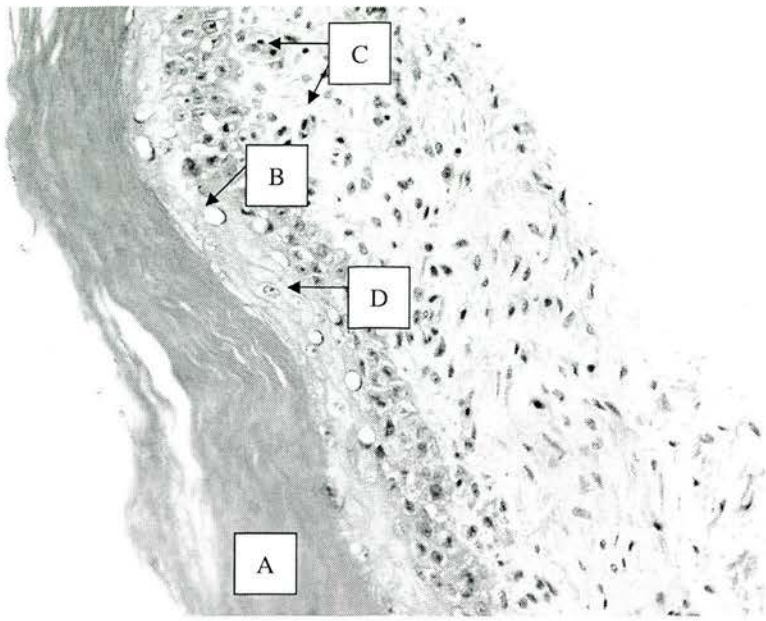

Figure 2. *Early FPD lesion in a 1-week-old turkey foot pad (histopathological score 3; magnification × 40). A = loose and excess keratin; B = hydropic degeneration; C = increased blood vessel density in dermis; D = epidermal acanthosis.*

keratin, hyperkeratinisation, keratin formed into 'horned pegs', epidermal hyperplasia, epidermal acanthosis, hydropic degeneration in the epidermis, and an increase in the number of blood vessels in the dermis.

Affected turkeys

Turkeys aged one week exhibited a few minor cellular changes, including hydropic degeneration, acanthosis and an increase in blood vessels in the dermis. The surface keratin showed very minor abnormalities, either loosely packed, in 'horned pegs' or with areas of compressed keratin. The epidermis was normal. There was no evidence of any inflammatory cells within the sample (Figure 2).

At 2 and 3 weeks, affected turkeys (Figure 1(b)) exhibited the cellular changes that occurred at one week, but also showed areas of compressed keratin filled with necrotic cells. This compressed keratin either lay in a layer above the normal keratin on the surface or replaced normal keratin completely. A 1-week-old bird showed evidence of a lesion with a ruptured epidermis and an influx of inflammatory cells from the dermis (Figure 3). The classification of a lesion, as used in this study, was that a ruptured epidermis, either with or without eroded surface keratin, was recorded as a fully developed lesion (Table 1).

Turkeys aged 4 and 5 weeks exhibited a greatly increased thickness of the keratin, which was more likely than not to have formed into 'horned pegs'. The epidermis was also thickened and had formed into rete pegs, with a great deal of folding of the epithelial layer. There was evidence of a few inflammatory cells in the dermis, but not of any great density. These inflammatory cells included large round purple stained cells with granulated nuclei that were interpreted as macrophages. Pink stained cells with polymorphic nuclei were also present and these were diagnosed as heterophils.

At 6 and 7 weeks, some turkeys showed a dense mass of inflammatory cells within the dermis, with an intact epidermis, but in others the epidermis had ruptured (Figure 3). Compressed keratin lay on the surface above the cavity caused by the ruptured epidermis. There was no normal keratin above the lesion, except at the edges of the lesion, where it had not been eroded. The dermis was a dense mass of inflammatory cells (mostly heterophils), associated with a marked increase in blood vessels, some of which were congested. The accumulation of inflammatory cells had resulted in a rupture of the epidermis. Some cells were located between the epidermis and the compressed keratin on the surface of the sample.

After 8 to 10 weeks, the lesions had become more severe. In some cases the compressed keratin on the surface of the lesion had been totally eroded (since the epidermis was no longer present), exposing a dermis completely congested with inflammatory cells, that were mostly heterophils. The dermis also showed evidence of apoptotic nuclei, and some inflammatory cells

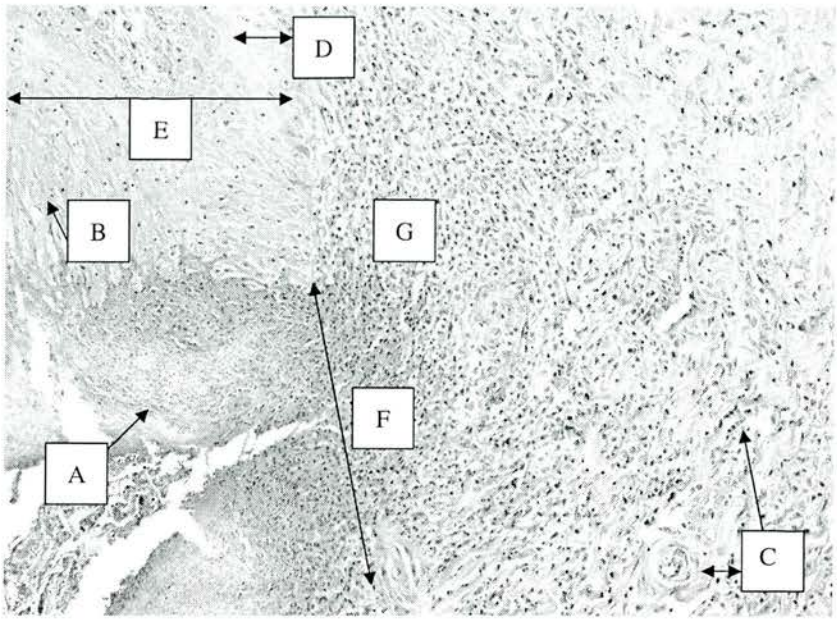

Figure 3. *Advanced FPD lesion in a 6-week-old turkey foot pad (histopathological score 7; magnification × 20). A = compressed and eroded surface keratin; B = hydropic degeneration; C = increased blood vessel density in dermis; D = epidermal acanthosis; E = epidermal hyperplasia; F = ruptured epidermis; G = inflammatory cells in dermis.*

even in deeper layers. These turkeys were also highly likely to have more than one lesion per foot pad, with as many as 5 lesions recorded in a single sample.

At 21 weeks of age, the turkeys showed more necrosis of cells within the epidermis, resulting in a larger lesion, but they also showed an increase in the amount of compressed keratin (full of necrotic cells) on the surface of the samples. The surface of the foot pad often appeared black due to an increase in the amount of necrotic material. There was also an increased number of heterophils and macrophages within the dermis. These older turkeys showed evidence of chronic inflammation, rather than the acute inflammation of the younger turkeys. The epidermis sometimes reformed over the site of rupture, yet the dermis still contained a high density of inflammatory cells. In such samples, the epidermis showed evidence of acanthosis, and was greatly thickened, covering the dermis, which was full of heterophils and macrophages.

DISCUSSION

Until now, it has been thought within the industry that lesions usually appear in commercial turkeys aged 8 to 12 weeks. The present experiment has shown that cellular changes occur, and progress relatively quickly, from an earlier age than previously thought. In older turkeys, the severity, size and number of lesions in affected foot pads was greater than in younger turkeys. A fully developed lesion (that is, where the integrity of the epidermis has been lost, see Table 1) was observed when the turkeys were just 3 weeks old, becoming increasingly more common with age. By 6 weeks of age, over two-thirds of turkeys that showed external evidence of a foot pad lesion showed histopathological evidence of a lesion as well. The basic histopathological findings did not change a great deal after 6 weeks of age. The surface keratin became more eroded and there was an increase in cell necrosis in the dermis and epidermis. In older turkeys, acute inflammation became chronic, as the skin of the foot pad attempted to compensate for this continued insult. The keratin and epidermis may have been repaired, but a large number of inflammatory cells (heterophils and macrophages) remained in the dermis. The surface of lesions still appeared black as a result of necrosis. It was interesting to note that turkeys with no external signs of lesions did in fact show signs of microscopic cellular changes after the age of 4 weeks. This suggests that these turkeys were beginning to develop FPD lesions at a later age than the obviously affected turkeys. Differences between individuals may be associated with genetic factors related to skin structure or behavioural differences, for example.

Externally, the lesions began as small hard discolourations of the skin, progressing into cracks, haemorrhaging and scabs on the metatarsal and digital pads along with acute inflammation and necrosis of the epidermis in mature lesions as reported by Whitehead (1977) and

Greene *et al.* (1985). At a cellular level, hyperkeratosis of the surface keratin and epithelial hyperplasia were often observed, which is consistent with previous results (Whitehead, 1990). Plant material and bacteria were usually present on the surface, but not in deeper layers, suggesting that FPD is not a response to bacterial invasion. The number of lymphocytes and granulocytes, as well as the number of lymph follicles in the dermis adjacent to the lesions increased.

Mild lesions were accompanied by an infiltration of heterophils into the *stratum germinativum* and defects in keratin formation, as observed by Martland (1984). In more severe lesions, heterophils filtered into the dermis, sub-epidermis and epidermis. There was also evidence of necrotic cells in the keratin layer. Vacuoles were observed in the epidermal cells. At the centre of the lesion, the epidermis and keratin were often completely destroyed, being replaced by a mass of inflammatory cells (Greene *et al.*, 1985). Dermal capillaries were congested and dilated, and epidermal hyperplasia at the edges of the lesion occurred. The keratin layer had more obvious defects in formation and often formed 'horned pegs' as noted by Martland (1984), Greene *et al.* (1985) and Whitehead (1990). As the turkeys aged, fewer superficial lesions were observed, whilst the number of ulcers that spread into the dermis increased, as noted in a comparison of 6- and 14-week-old turkeys (Platt *et al.*, 2001).

Foot pad lesions have also been studied in broiler chickens but, as yet, no one is certain of the cause in chickens or turkeys. Factors in the external environment such as the condition of the litter (Harms and Simpson, 1977; Harms *et al.*, 1977; Martland, 1984; McIlroy *et al.*, 1987; Lumb, 2002) and components of the diet (Harms and Simpson, 1975, 1977; Harms *et al.*, 1977; Whitehead and Bannister, 1981; Platt *et al.*, 2001) have been associated with the development of FPD. It is also possible that FPD is caused by internal factors such as the structure of the skin, rapid growth and high body weight (as increased body weight may increase the pressure exerted on the foot pad), or the sex of the turkeys (as males tend to be heavier when fully grown, and therefore would exert more pressure on the foot pads than females). Whilst many individual factors have been reported to affect the prevalence and development of FPD, the condition is likely to be multifactorial in origin. Further experimental work is required to determine exactly what causes FPD, and why the pathology develops.

These results were taken from four different farms, all managed in an identical manner within a consecutive 2 d period. All farms had the same timetable for changing litter, ventilation systems and maintenance, feeding and watering systems, resulting in similar litter quality in each farm and house visited. All the turkeys within each farm were checked for adequate weight gain by the farm staff, and were within guidelines set within the industry, so the weight of the turkeys should not have affected foot pad lesions. All farms were owned and managed by one of the two major turkey producers in the UK. This producer is responsible for a large proportion of commercially reared turkeys within the UK, suggesting that the samples taken were representative of the UK turkey industry as a whole. The fact that the turkeys sampled at 4 weeks were female should not affect foot pad lesion results. Female and male turkeys of this age are similar in terms of weight gain, size and level of development. Therefore, if weight is a factor that may affect FPD development, this should not have had an effect at this age. All turkeys sampled were physically injured in some way, but this should not affect foot pad status. The turkeys sampled had been head or vent pecked, or suffered broken wings. There is a possibility that injured turkeys may have been weaker and smaller, resulting in their being pecked. If these turkeys were smaller and less robust than others this may have made them more susceptible to foot pad lesions, but this is unlikely. Such injuries are not believed by the authors to have any effect on the development of FPD. These experimental results are from a sample of turkeys taken to assess the development of turkey FPD. Since only two turkeys were taken from each category, that is, two affected and two non-affected birds from each age group, it cannot be claimed that all turkeys of a certain age on these farms will exhibit lesions of the same severity as the results obtained within this experiment. These results can however be used to expose novel information as to how the condition of FPD develops.

In conclusion, we have shown that there was cellular evidence of an inflammatory response associated with FPD in commercial turkeys, at an earlier age than previously reported, and that the changes apparently proceed rapidly to mature lesions in less than 3 weeks. All turkeys sampled at 4 weeks and over exhibited histopathological changes associated with FPD, even in foot pads that appeared normal externally. Turkeys aged 6 to 21 weeks with foot pad lesions showed increasing levels of necrosis. Foot pad lesions had already occurred in these turkeys, and the area of necrosis simply spread. On the basis of the present experiment, the authors hypothesise that FPD may be occurring within turkey flocks in the UK industry at an earlier age than previously thought. The data obtained can be used to identify the manner in which this

condition develops, although further exploration is required.

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